UTTARAKHAND OPEN UNIVERSITY
Teenpani Bypass Road, Transport Nagar, Haldwani - 263 139
Phone No. : (05946) - 286002, 286022, 286001, 286000
Toll Free No. : 1800 180 4025
Fax No. : (05946) - 264232, email : <info@uou.ac.in>
http://www.uou.ac.in

ENSE - 653

Environmental Microbiology and Biotechnology

ENSE - 653

Environmental Microbiology and Biotechnology

Department of Forestry and Environmental Science
School of Earth and Environmental Science

Uttarakhand Open University
Haldwani, Nainital (U.K.)

ENSE 653

Environmental Microbiology and Biotechnology

UTTARAKHAND OPEN UNIVERSITY

SCHOOL OF EARTH AND ENVIRONMENTAL SCIENCE

University Road, Teenpani Bypass, Behind Transport Nagar, Haldwani - 263 139

Phone No. : (05946) - 286002, 286022, 286001, 286000

Toll Free No. : 1800 180 4025, Fax No. : (05946) - 264232,

e-mail: info@uou.ac.in, Website: http://www.uou.ac.in

Board of Studies

Programme Coordinator

Dr. H.C. Joshi
Department of Forestry and Environmental Science,
SoEES, Haldwani, Nainital (U.K.)

Editors

H.C. Joshi, Beena T. Fulara, Krishna K. Tamta,
Neha Tiwari, Preeti Pant, Deepti Negi & Khashti Dasila

Department of Forestry and Environmental Science
Uttarakhand Open University, Haldwani (U.K.)

Unit Writers	Unit No.
Dr. Preety Pant, Department of Food Technology, Swamy Dayanand University, Sagar (M.P.)	1, 2, 3, 4
Dr. Anuj Kumar Sharma, Department of Botany, Swami Vivekananda College of Education, Roorkee (U.K.)	5,6
Dr. Vikas Saini, Deptt. of Environmental Sciences, Swami Vivekananda College of Education Roorkee, (U.K.)	7, 12
Adapted from e-PG Pathshala, Paper No. 05-Environmental Biotechnology, module nos. 9, 11, 22 (course credit Nimisha Jadon, Rajeev Jain)	8
Dr. Sushil Bhadula, Head, Department of Zoology, Swami Vivekananda College of Education Roorkee, Uttarakhand	9,10,11, 13

Cover Page Design and Format Editing

Dr. Krishna Kumar Tamta, Dr. Beena Tewari Fulara, Dr. H.C. Joshi
Department of Forestry and Environmental Science, Uttarakhand Open University, Haldwani

Title : Environmental Microbiology and Biotechnology
ISBN : XXXX-XXXX
Copyright : Uttarakhand Open University
Edition : 2024 (Restricted distribution only).
Published By : Uttarakhand Open University, Haldwani, Nainital (U.K.)
Printed at :

Table of Contents

Unit 1: Microbes and Environment

Unit Structure

1.0 Learning Objectives

After studying this unit you would be able to:

1. Explain what are microorganisms and why these are taken up as a unit of study
2. Identify the various types of microorganisms
3. Explain the occurrence and distribution of microorganisms
4. Describe the diversity of microorganisms
5. Explain the ecological significance of microorganisms

1.1 Introduction

Microorganisms are the organisms which can't be seen by necked eyes. These only can be seen with the help of microscope. Microbes may exist as single-celled form or in a colony of cells. [80]

The probable existence of microorganisms was assumed from prehistoric times, such as in Jain scriptures from 6th century BC India and the 1st century BC book On Agriculture by Marcus Terentius Varro.

The scientific study of microorganisms is known as microbiology. First of all Antonie van Leeuwenhoek observed these under the microscope in 1670s. In 1850s, Louis Pasteur found that microorganisms caused spoilage of food, debunking the theory of spontaneous generation. In the 1880s, Robert Koch revealed that microorganisms caused the diseases tuberculosis, cholera and anthrax.

Microorganisms consist of all unicellular organisms. These are quite diverse. Carl Woese identified three domains of life. All the Archaea and Bacteria are microorganisms. These were earlier grouped together in the two domain system as Prokaryotes, the other being the eukaryotes. The third domain Eukaryota includes all multicellular organisms and many unicellular protists and protozoans. Some protists are linked to animals and some to green plants. Many of the multicellular organisms are microscopic, that is micro-animals, as some fungi and algae.

Microorganisms live in nearly every habitat from the poles to the equator, deserts, geysers, rocks and the deep sea. Some are adapted to extremes such as very hot or very cold conditions, others to high pressure and a few to high radiation environments such as Deinococcus radiodurans. Microorganisms also make up the microbiota, found in and on all multicellular organisms. According to a report, the most primitive direct proof of life on earth is that 3.45-billion-year-old Australian rocks once contained microorganisms.[1][2]

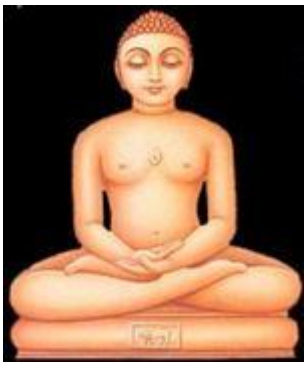
Microbes are important as being useful as well as harmful for human being. These are used to ferment foods, treat sewage; and produce fuel, enzymes and other bioactive compounds. They are important to use in biological war and bioterrorism. These are an essential constituent of fertile soils. In the human body microorganisms are necessary gut flora. Some microorganisms are harmful pathogens, responsible for many contagious diseases.

1.3 Occurrence and Distribution (Discovery)

Antonie van Leeuwenhoek, the first to study microorganisms, using simple microscopes of his own design.

Lazzaro Spallanzani showed that boiling a broth stopped it from decay.

1.3.1 Ancient



Vardhmana Mahavira postulated the life of microscopic creatures in the 6th century BC.

The microorganisms were discovered in the 17th century. By the fifth century BC, the Jains of present-day India postulated the survival of minute organisms called nigodas.[3] The characteristics of nigodas are as, they born in clusters; exist in all places, including the bodies of plants, animals, and people; and their life lasts only for fraction of a second.[4]

According to the Jain saint Mahavira, the humans destroy these nigodas on a huge scale, when they eat, breathe, sit and move.[3] Many modern Jains emphasize that Mahavira's teachings signify the survival of microorganisms as discovered by modern science.[5]

Marcus Terentius Varro, a Roman scholar, indicated the possibility of diseases dispersed by unseen organisms. He called the invisible creatures animalcules, in a 1st-century BC book titled On Agriculture. He warns alongside locating a homestead in close proximity to a swamp:[6] Certain microscopic creatures may bred there. These creatures cannot be seen by the necked eyes, which may float in the air and come into the body through the mouth and nose and cause severe diseases.[6]

In The Canon of Medicine (1020), Avicenna recommended that tuberculosis and other diseases might be infectious.[7][8]

1.3.2 Early modern

Akshamsaddin, Turkish scientist, mentioned the microbe in his work Maddat ul-Hayat (The Material of Life) about two centuries prior to Antonie Van Leeuwenhoek's discovery through experimentation.

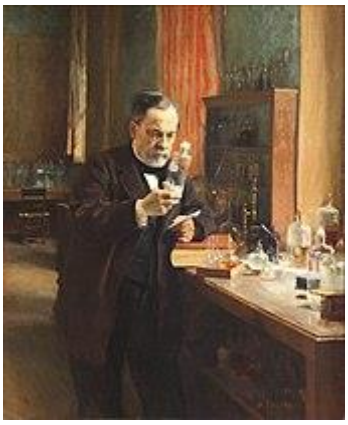
It was said that it is mistaken to presume that diseases emerge one by one in humans. Disease infects by dispersal from one person to another. This infection occurs through seeds. These are so minute to be seen but are alive.[9][10]

Girolamo Fracastoro (1546) anticipated that transferable seed like entities caused epidemic diseases. These might spread infection by direct or indirect contact, or even without contact over long distances.[11]

Antonie Van Leeuwenhoek is known as the father of microbiology. Firstly he (1673) discovered, observed, described, studied and conducted scientific experiments with microorganisms He uses simple single-lensed microscopes designed by himself.[12][13][14][15]

Robert Hooke also used microscope to study microbial life in the form of the fruiting bodies of moulds. He made drawings of studies in his book Micrographia (1665) and coined the term cell.[16]

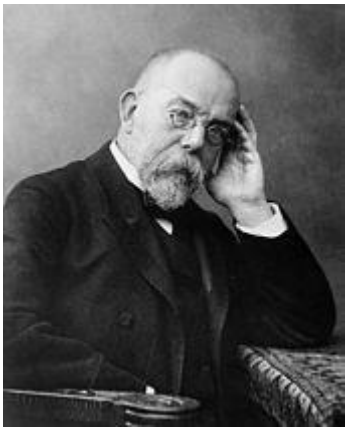
1.3.3 19th century



Louis Pasteur showed that Spallanzani's findings held even if air could enter through a filter that kept particles out.

Louis Pasteur (1822–1895) disproved the theory of spontaneous generation and supported the germ theory of disease.[17] He boiled the broth and ensured that no microorganisms survived within the broths at the start of his experiment. He exposed the boiled broth to the air, in vessels. One vessel contained a filter to check particles from passing through to the growth medium. Another one was without a filter, but with air allowed in via a curved tube, so dust particles would resolve and could not come in contact with the broth.

Nothing grew in the broths in the course of Pasteur's experiment. He concluded that the living organisms that grew in such broths came from external environment, as spores on dust, rather than spontaneously generated inside the broth.



Robert Koch showed that microorganisms caused disease.

Robert Koch (1876) explained that microorganisms can cause disease, by establishing a fundamental link between a microorganism and a disease, now known as Koch's postulates.[18] He found that the infected blood of cattle with Anthrax always had huge numbers of Bacillus anthracis. By injecting a small sample of infected blood to the healthy animal can cause the healthy animal to become sick. Anthrax could be transmitted from one animal to another.

He also found that he could grow the bacteria in a nutrient broth and by injecting it into a healthy animal cause illness. Based on these experiments he gave Koch's postulates. Even though these postulates cannot be functional in all cases, yet have historical importance to develop scientific thought and are still being used today.[19]

In 1860s a third kingdom was named by the discovery of microorganisms such as Euglena, as it had some characteristics of both, animal and plant kingdoms. They were photosynthetic like plants, but motile like animals. In 1860 John Hogg called this the Protoctista, and in 1866 Ernst Haeckel named it the Protista.[20][21][22]

The true wideness of microbiology was discovered by the work of Martinus Beijerinck and Sergei Winogradsky late in the 19th century .[23] Beijerinck discovered viruses and the development of enrichment culture techniques.[24] He established the fundamental principles of virology by working on the Tobacco Mosaic Virus.

Winogradsky firstly developed the theory of chemolithotrophy. He revealed the vital role of microorganisms in geochemical processes.[25] He firstly isolated and described

both nitrifying and nitrogen-fixing bacteria.[23] French-Canadian microbiologist Felix d'Herelle co-discovered bacteriophages and was one of the earliest applied microbiologists.[26]

Self -Assessment 1

1. What do you understand by microbes?

   _____

   _____

   _____

   _____

   _____

2. Who described the three domains of life?

   _____

   _____

   _____

   _____

   _____

3. Write a short note on three domains of life.

   _____

   _____

   _____

   _____

   _____

4. Are microorganisms useful or harmful? Write a short note.

   _____

   _____

   _____

   _____

   _____

5. What are Nigodas? Explain in short.

_____

_____

_____

_____

_____

6. Who is known as the father of microbiology? Who wrote the book Micrographia?

_____

_____

_____

_____

_____

6. Who disproved the theory of spontaneous generation and supported the germ theory of disease? Write his experiment also.

_____

_____

_____

_____

_____

7. Who explained that microorganisms can cause disease? Explain shortly his experiments also.

_____

_____

_____

_____

_____

8. Who firstly developed the theory of chemolithotrophy?

_____

_____

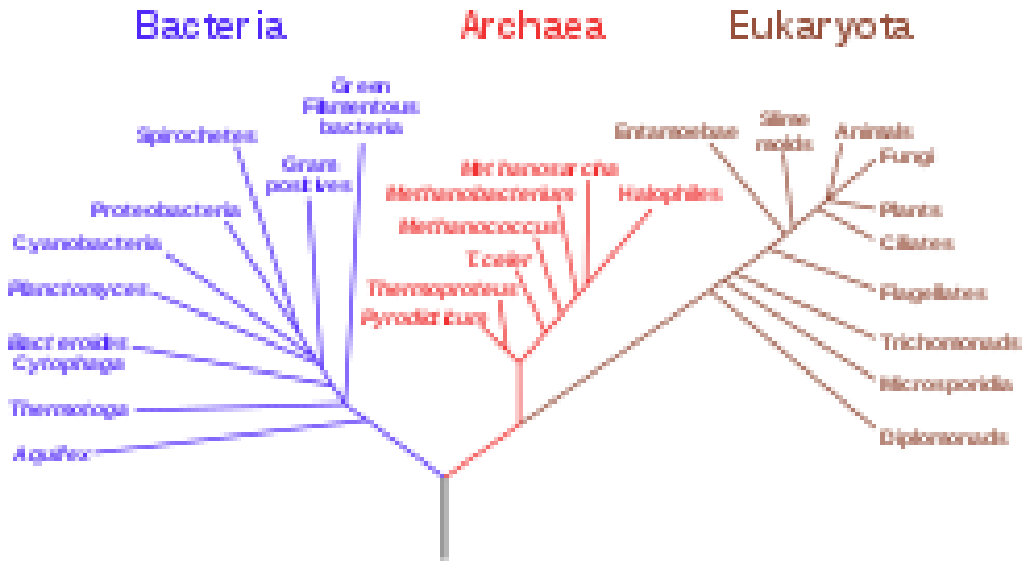
_____

_____

_____

1.4 Diversity

Microorganisms can be found approximately everywhere on Earth. Bacteria and Archaea are roughly constantly microscopic, whereas a number of eukaryotes are also microscopic, together with most protists, some fungi, as well as some micro-animals and plants. Viruses are usually regarded as non living and thus not considered as microorganisms. The study of viruses is called virology.[27][28][29]



Carl Woese's 1990 phylogenetic treebased on rRNA data shows the domains of Bacteria, Archaea, and Eukaryota. All are microorganisms except some eukaryote groups.

Approximately 3–4 billion years ago, single-celled microorganisms developed on earth were the first forms of life.[30][31][32] Further evolution was slow.[33] Approximately 3 billion years in the Precambrian era, (much of the history of life on Earth), all organisms were microorganisms.[34][35] Bacteria, algae and fungi have been identified in yellowish-brown that

is 220 million years old, which shows that the morphology of microorganisms has changed little since the Triassic period.[36]

Volcanic eruptions from the Siberian Traps brought nickel, which might accelerated the evolution of methanogens towards the end of the Permian–Triassic extinction.[37]

Most microorganisms can reproduce quickly and apt to a fairly fast rate of evolution. Conjugation, transformation and transduction processes are used by bacteria for exchange of genes, even between widely different species.[38]

Microorganisms evolve speedily via natural selection as the result of gene transfer together with a high mutation rate. It also helps to survive in new environments and to abide environmental stresses. [39]

Japanese scientists (2012) discovered a probable intermediary form of microbe between a prokaryote and a eukaryote. Parakaryon myojinensis, an exceptional microbe, larger than a usual prokaryote, but with nuclear material enclosed in a membrane as in a eukaryote. Endosymbionts were also present. It showed a stage of development from the prokaryote to the eukaryote.[40][41]

1.4.1 Archaea

Carl Woese's three-domain system showed that Archaea were the first domain of life. They were prokaryotic unicellular organisms. Prokaryotes do not have cell nucleus or other membrane bound-organelles.

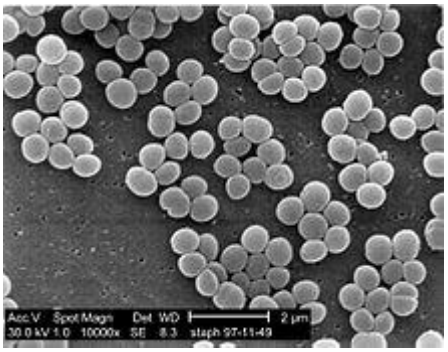
Woese, a microbiologist, in 1990 proposed the three-domain system that divided living things into bacteria, archaea and eukaryotes,[42] and thus split the prokaryote domain.

Archaea vary from bacteria in both their genetics and biochemistry. For example, bacterial cell membrane is made from phosphoglycerides with ester bonds, while archaean membranes are made of ether lipids.[43] Archaea were initially described as extremophiles existing in extreme environments, such as hot springs. They are known to be found in all types of habitats.[44] Archaea are very commonly found in the environment, as Crenarchaeota is the most common form of life in the ocean, dominating ecosystems below 150 m in depth.[45][46] These organisms are also frequent in soil and play an essential role in ammonia oxidation.[47]

Archaea and bacteria commonly make the most varied and abundant group of organisms on Earth and reside in basically all environments where the temperature is below +140 °C. They are found in water, soil, air, as the micro biome of an organism, hot springs and even deep underneath the Earth's crust in rocks.[48] The number of prokaryotes is probable to be around five million trillion trillion, or 5×10^{30}, accounting for at least half the biomass on Earth.[49]

The biodiversity of the prokaryotes is unidentified, but may be very large. It is estimated (2016) that perhaps 1 trillion species are on the planet, of which mainly would be microorganisms. At present, only one-thousandth of one percent of that total have been described.[50]

1.4.2 Bacteria



Staphylococcus aureus bacteria magnified about 10,000 x

Bacteria are prokaryotic organisms. These are unicellular, don't have cell nucleus and other membrane bound organelles. Bacteria are microscopic, with a few exceptionally unusual examples, such as Thiomargarita namibiensis.[51] Bacteria function and reproduce as individual cells, but they can frequently aggregate in multicellular colonies.[52] Some species such as myxobacteria can amassed into composite swarming structures, working as multicellular groups as part of their life cycle.[53] They may form clusters in bacterial colonies such as E.coli.

Bacterial genome is generally a circular bacterial chromosome i.e. a single loop of DNA, even though they can also harbor small pieces of DNA called plasmids. These plasmids can be transferred between cells through bacterial conjugation. Bacteria have an enclosing cell wall, which provides strength and rigidity to their cells. Reproduction by binary fission or

occasionally by budding. But meiotic sexual reproduction is not undergone. A horizontal gene transfer process may present in many species of bacteria which is known as natural transformation.[54] In this process bacterial species can transfer DNA between individual cells.

For survival some species form extremely tough spores. Under most favorable conditions bacteria can develop extremely quickly and their numbers can be doubled as rapidly as every 20 minutes.[55]

1.4.3 Eukaryotes

Eukaryotes are observable to the naked eye. Most living things are eukaryotes, together with humans. Though, a big number of eukaryotes are also microorganisms. Eukaryotes have cell organelles such as cell nucleus, Golgi apparatus and mitochondria.

DNA (Deoxyribonucleic acid) is arranged in complex chromosomes in nucleus.[56] Fundamental metabolism takes place in mitochondria as citric acid cycle and oxidative phosphorylation.

Eukaryotes evolved from symbiotic bacteria and keep a trace genome. Similar to bacteria, plant cells have cell walls. They have chloroplasts which produce energy from light by photosynthesis, and were also initially symbiotic bacteria.[57]

Unicellular eukaryotes have single cell during their life cycle. Microbial eukaryotes can be either haploid or diploid, and some organisms have several cell nuclei.[58]

They have asexual reproduction, as mitosis under favorable conditions. However, under unfavorable conditions, like nutrient limitations and other conditions related with DNA damage, sexual reproduction is found by meiosis and syngamy.[59]

1.4.4 Protists

***Euglena mutabilis*, a photosynthetic flagellate**

Protists are eukaryotes, generally unicellular and microscopic. This is a vastly diverse group of organisms that are not easily be classified [60][61]. Numerous algae are multicellular protists. Slime mold's exceptional life cycle switch between unicellular, colonial, and multicellular forms.[62] Various species of protists are unknown as only a small section has been recognized. Protist diversity is high in oceans, deep sea-vents, river sediment and an acidic river, signifying that many eukaryotic microbial communities may yet be revealed.[63][64]

1.4.5 Fungi

Fungi are several unicellular organisms, such as baker's yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*). Some pathogenic fungi such as yeast (*Candida albicans)*, can go through phenotypic switching. They grow as single cells in some environments, and filamentous hyphae in others.[65]

1.4.6 Plants

The green algae are a large group of photosynthetic eukaryotes that consist of many microscopic organisms. Even though some green algae are classified as protists, others such as charophyta are classified with embryophyte plants, which are the most common group of land plants.

Algae can develop as single cells or in long chains of cells. The green algae consist of unicellular and colonial flagellates. Generally but not always they have two flagella per cell.

A range of colonial, coccoid, and filamentous forms are also found. Charales, the algae most strongly correlated to higher plants, have the cells distinguished into numerous diverse tissues within the organism. There are about 6000 species of green algae.[66]

Self- Assessment 2

1. Which microorganisms are capable to freely exchange genes through conjugation, transformation and transduction?

   _____

   _____

   _____

   _____

   _____

2. According to Carl Woese's three-domain system which microorganisms was the first domain of life?

   _____

   _____

   _____

   _____

   _____

3. Which microorganisms showed a stage of development from the prokaryote to the eukaryote?

   _____

   _____

   _____

   _____

   _____

4. Which bacteria can be amassed into composite swarming structures?

_____

_____

_____

_____

_____

5. The life cycle of which mold switches between unicellular, colonial, and multicellular forms?

_____

_____

_____

_____

_____

6. How many species of green algae are found?

_____

_____

_____

_____

_____

1.5 Ecological Significance

Microorganisms are found in approximately each and every habitat of nature, together with unreceptive environments such as the North and South poles, deserts, geysers, and rocks. They also consist of all the marine microorganisms of the oceans and deep sea.

Some types of microorganisms have modified as according to extreme environments and sustained colonies; so acknowledged as extremophiles. Extremophiles have been isolated from rocks as about 7 kilometers under the Earth's surface,[67] it has been suggested that the quantity of organisms living under the Earth's surface is equivalent with the quantity of life on or above the surface.[48]

Extremophiles are known to continue to exist for a prolonged time in a vacuum. They may be greatly resistant to radiation, so that they may stay alive in space.[68]

Microorganisms may be symbiotic with other larger organisms. Some of which are mutually advantageous (mutualism), though others can be harmful to the host organism (parasitism). Some microorganisms can cause disease in a host, known as pathogens, so sometimes referred to as *microbes*.

Mutualism

Mutualism is a relationship either between microbial species or between microbial species and humans, in which both the organisms are benefited.[82] Syntropy, also known as cross-feeding is one example of it.[81] Lichen is an example of a symbiotic organism.[83]

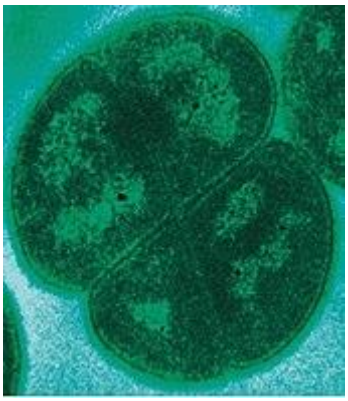
Amensalism

Amensalism (antagonism) is a type of symbiotic relationship in which one organism is harmed whereas the other remains unaffected.[82] Example of it is relationship between *Lactobacillus casei* and *Pseudomonas taetrolens*.[84] The growth of *Pseudomonas taetrolens is* inhibited and decreased production of lactobionic acid (its main product). It is mainly due to the byproducts formed by *Lactobacillus casei* during the production of lactic acid.[85] On the other hand, there is no difference in the activities of *Lactobacillus casei*.

Microorganisms take part in significant roles in biogeochemical cycles of earth as in decomposition and nitrogen fixation.[69]

By using regulatory networks bacteria are adapted to approximately all environmental slot on earth.[70][71] Bacteria attain regulation of gene expression by utilizing a network of interactions amongst various types of molecules including DNA, RNA, proteins and metabolites . In bacteria, the prime purpose of regulatory networks is to control the response to environmental changes, as nutritional status and environmental stress.[72] A composite association of networks permits the microorganism to organize and incorporate numerous environmental signals.[70]

Extremophiles



A tetrad of *Deinococcus radiodurans*, a radioresistant extremophile bacterium

Extremophiles are microorganisms that have modified themselves to can survive and constant flourish in extreme environments, usually lethal to most life-forms. Thermophiles and hyperthermophiles flourish in high temperatures, as high as 130 °C (266 °F)[73] . Psychrophiles flourish in extremely low temperatures, as low as −17 °C (1 °F). [74]

Halophiles such as *Halobacterium salinarum* (an archaean) flourish in high salt conditions, up to saturation.[75] Alkaliphiles flourish in an alkaline pH of about 8.5–11.[76] Acidophiles can flourish in a pH of 2.0 or less.[77] Piezophiles flourish at very high pressures: up to 1,000–2,000 atm, down to 0 atm as in a vacuum of space.[78]

Some extremophiles such as *Deinococcus radiodurans* are radioresistant [79] resisting radiation exposure of up to 5k Gy. Extremophiles are significant in extending global life into a large amount of the Earth's hydrosphere, crust and atmosphere.

1.5.1 Microbial ecology and Role of microorganism in ecosystem

Microbial ecology [86]

- It is the association of microorganisms with one another and with their environment. Microorganism impacts the whole biosphere. They are present almost in all part of planet earth as well as in extreme conditions such as acidic lakes to deep ocean and from frozen environment to hydrothermal vents.

- Ecological significance includes all microbial processes take place in soil, water and air. It is not only concern with particular microenvironment where the microorganisms in fact working but with the broader consequence of microbial existence and action also.
- It also includes microbial biodegradation of domestic, agricultural and industrial wastes and also sub-surface pollution in soil, sediments and marine environments.

Role of microorganism in ecosystem

1. Produce Oxygen in environment

- Approximately all of the oxygen formed in earth today occurs by bacteria in prehistoric era.

The majority of the oxygen producers are cyanobacteria (blue green algae) in ocean.

2. Reprocess nourishment stored in organic matters to inorganic form:

- The minerals such as N, P, K etc are released by the microbial breakdown of organic matters. These minerals are bound up and made available for producers (green plants) to utilize. Prime productivity of ecosystem would bring to an end if the recycling or reprocess is not done.
- Fungi are the most significant decomposers of plant materials in soil followed by bacteria.

3. Fix atmospheric Nitrogen into useable form:

- The nitrogen fixing bacteria (*Rhizobium*, *Cyanobacteria* etc) fix atmospheric nitrogen into useable form.
- The nitrogen gas is 78% of the earth's atmosphere, but unavailable to most of the organisms. It is converted to a biologically available form by the microorganisms.

4. Microorganisms help plant roots to surplus to nutrition in soil:

- Roots of the plants use available nutrition of soil. It creates a zone of nutrition depletion in the region of their surface.
- A group of fungi known as mycorrhiza coupled with rood hairs of plants and helps in efficient absorption of nutrition in soil.

5. Allows herbivore animals to get nutrition from poor quality food:

- In ruminant animals the digestive enzymes in gut can't digest ingested food rich in particularly cellulose, lignin etc. The rumen (stomach) of these animals contains various bacteria, fungi and protozoa that carry out fermentation and help in the digestion of these complex carbohydrates.

https://www.onlinebiologynotes.com/microbial-ecology-and-role-of-microorganism-in-ecosystem/

Self-Assessment 3

1. Write a short note on the habitat of microorganisms.

_____

_____

_____

_____

2. What are extremophiles? Explain in short.

_____

_____

_____

_____

3. At what temperature Thermophiles, Psychrophiles and Hyperthermophiles flourish?

_____

_____

_____

_____

4. In which conditions Halophiles, Alkaliphiles and Piezophiles flourish?

_____

_____

_____

_____

Summary

In this chapter you have learnt that:

- Microorganisms are the organisms which can't be seen by necked eyes. These only can be seen with the help of microscope.
- The scientific study of microorganisms is known as microbiology.
- Antonie Van Leeuwenhoek is known as the father of microbiology.
- Microorganisms live in nearly every habitat from the poles to the equator, deserts, geysers, rocks and the deep sea.
- Some types of microorganisms have modified as according to extreme environments and sustained colonies; so acknowledged as extremophiles.
- Microbes may exist as single-celled form or in a colony of cells.
- Microorganisms consist of all unicellular organisms.
- Bacteria and archaea are roughly constantly microscopic, whereas a number of eukaryotes are also microscopic, together with most protists, some fungi, as well as some micro-animals and plants.
- Viruses are usually regarded as non living and thus not considered as microorganisms.
- Microorganisms may be symbiotic with other larger organisms.
- Some of which are mutually advantageous (mutualism), though others can be harmful to the host organism (parasitism).
- Some microorganisms can cause disease in a host, known as pathogens, so sometimes referred to as *microbes*.
- Microorganisms take part in significant roles in biogeochemical cycles of earth as in decomposition and nitrogen fixation.

Terminal Questions

1. Explain in detail about the discovery of microorganisms in different era.

2. Write down the theories given by Louis Pasture and Robert Koch about the discovery of microbes.

3. Write in detail about the habitat of different kinds of microbes.

4. Explain the role of microbes in ecosystem.

5. Write a note on microbial ecology.

6. What is microbiology? Are microbes useful or harmful, explain in detail.

1.8 Answers

Self-assessment 1

1. Refer to section 1.1
2. Refer to section 1.1
3. Refer to section 1.1
4. Refer to section 1.1
5. Refer to section 1.3.1
6. Refer to section 1.3.1
7. Refer to section 1.3.3
8. Refer to section 1.3.3
9. Refer to section 1.3.3

Self-assessment 2

1. Refer to section 1.4
2. Refer to section 1.4.1
3. Refer to section 1.4
4. Refer to section 1.4.2
5. Refer to section 1.4.4
6. Refer to section 1.4.6

Self-assessment 3

1. Refer to section 1.5
2. Refer to section 1.5
3. Refer to section 1.5
4. Refer to section 1.5

Terminal Questions

1. Refer to section 1.3
2. Refer to section 1.3.3
3. Refer to section 1.5
4. Refer to section 1.5
5. Refer to section 1.5.1
6. Refer to section 1.1 and 1.5

Reference

1. *Tyrell, Kelly April (18 December 2017).* "Oldest fossils ever found show life on Earth began before 3.5 billion years ago". University of Wisconsin–Madison. Retrieved 18 December 2017.

2. *Schopf, J. William; Kitajima, Kouki; Spicuzza, Michael J.; Kudryavtsev, Anatolly B.; Valley, John W. (2017).* "SIMS analyses of the oldest known assemblage of microfossils document their taxon-correlated carbon isotope compositions". PNAS. *115 (1): 53–58.* Bibcode*:2018PNAS..115...53S.* doi*:*10.1073/pnas.1718063115. PMC 5776830. PMID 29255053.
3. *Jeffery D Long (2013).* Jainism: An Introduction. *I.B.Tauris. p. 100.* ISBN 978-0-85771-392-6.
4. *Upinder Singh (2008).* A History of Ancient and Early Medieval India: From the Stone Age to the 12th Century. *Pearson Education India. p. 315.* ISBN 978-81-317-1677-9.
5. *Paul Dundas (2003).* The Jains. *Routledge. p. 106.* ISBN 978-1-134-50165-6.
6. Varro On Agriculture 1, xii Loeb
7. *Tschanz, David W.* "Arab Roots of European Medicine". *Heart Views. 4 (2). Archived from* the original *on 3 May 2011.*
8. *Colgan, Richard (2009).* Advice to the Young Physician: On the Art of Medicine. *Springer. p. 33.* ISBN 978-1-4419-1033-2.
9. Taşköprülüzâde: Shaqaiq-e Numaniya, v. 1, p. 48
10. Osman Şevki Uludağ: Beş Buçuk Asırlık Türk Tabâbet Tarihi (Five and a Half Centuries of Turkish Medical History). Istanbul, 1969, pp. 35–36
11. *Nutton, Vivian (1990). "The Reception of Fracastoro's Theory of Contagion: The Seed That Fell among Thorns?".* Osiris. *2nd Series, Vol. 6, Renaissance Medical Learning: Evolution of a Tradition: 196–234.* doi*:*10.1086/368701. JSTOR 301787.
12. *Leeuwenhoek, A. (1753). "Part of a Letter from Mr Antony van Leeuwenhoek, concerning the Worms in Sheeps Livers, Gnats, and Animalcula in the Excrements of Frogs". Philosophical Transactions. 22 (260–276): 509–18.* doi*:*10.1098/rstl.1700.0013.
13. Leeuwenhoek, A. *(1753). "Part of a Letter from Mr Antony van Leeuwenhoek, F. R. S. concerning Green Weeds Growing in Water, and Some Animalcula Found about Them". Philosophical Transactions. 23 (277–288): 1304–11.* doi*:*10.1098/rstl.1702.0042.
14. Lane, Nick *(2015).* "The Unseen World: Reflections on Leeuwenhoek (1677) 'Concerning Little Animal'". *Philos Trans R Soc Lond B Biol Sci. 370 (1666): 20140344.* doi*:*10.1098/rstb.2014.0344. PMC 4360124. PMID 25750239.
15. Payne, A.S. The Cleere Observer: A Biography of Antoni Van Leeuwenhoek, p. 13, Macmillan, 1970
16. *Gest, H. (2005). "The remarkable vision of Robert Hooke (1635–1703): first observer of the microbial world". Perspect. Biol. Med. 48 (2): 266–72.* doi*:*10.1353/pbm.2005.0053. PMID 15834198.
17. *Bordenave, G. (2003). "Louis Pasteur (1822–1895)". Microbes Infect. 5 (6): 553–60.* doi*:*10.1016/S1286-4579(03)00075-3. PMID 12758285.
18. The Nobel Prize in Physiology or Medicine 1905Nobelprize.org Accessed 22 November 2006.
19. *O'Brien, S.; Goedert, J. (1996). "HIV causes AIDS: Koch's postulates fulfilled". Curr Opin Immunol. 8 (5): 613–18.* doi*:*10.1016/S0952-7915(96)80075-6. PMID 8902385.
20. *Scamardella, J. M. (1999).* "Not plants or animals: a brief history of the origin of Kingdoms Protozoa, Protista and Protoctista" (PDF). *International Microbiology. 2: 207–221.*
21. *Rothschild, L. J. (1989). "Protozoa, Protista, Protoctista: what's in a name?". J Hist Biol. 22 (2): 277–305.* doi*:*10.1007/BF00139515. PMID 11542176.

22. *Solomon, Eldra Pearl; Berg, Linda R.; Martin, Diana W., eds. (2005).* "Kingdoms or Domains?". *Biology (7th ed.). Brooks/Cole Thompson Learning. pp. 421–7.* ISBN 978-0-534-49276-2.
23. *Madigan, M.; Martinko, J., eds. (2006). Brock Biology of Microorganisms (13th ed.). Pearson Education. p. 1096.* ISBN 978-0-321-73551-5.
24. *Johnson, J. (2001) [1998].* "Martinus Willem Beijerinck". *APSnet. American Phytopathological Society. Archived from* the original *on 2010-06-20.* Retrieved 2 May 2010.Retrieved from Internet Archive 12 January 2014.
25. *Paustian, T.; Roberts, G. (2009).* "Beijerinck and Winogradsky Initiate the Field of Environmental Microbiology". *Through the Microscope: A Look at All Things Small (3rd ed.). Textbook Consortia. § 1–14.*
26. *Keen, E. C. (2012). "Felix d'Herelle and Our Microbial Future". Future Microbiology. 7 (12): 1337–1339.* doi:10.2217/fmb.12.115. PMID 23231482.
27. *Lim, Daniel V. (2001). "Microbiology". eLS. John Wiley.* doi:10.1038/npg.els.0000459. ISBN 9780470015902.
28. "What is Microbiology?". *www.highveld.com*. Retrieved 2017-06-02.
29. *Cann, Alan (2011). Principles of Molecular Virology (5 ed.). Academic Press.* ISBN 978-0123849397.
30. *Schopf, J. (2006).* "Fossil evidence of Archaean life". *Philos Trans R Soc Lond B Biol Sci. 361 (1470): 869–885.* doi:10.1098/rstb.2006.1834. PMC 1578735. PMID 16754604.
31. *Altermann, W.; Kazmierczak, J. (2003). "Archean microfossils: a reappraisal of early life on Earth". Res Microbiol. 154 (9): 611–7.* doi:10.1016/j.resmic.2003.08.006. PMID 14596897.
32. Cavalier-Smith, T. *(2006).* "Cell evolution and Earth history: stasis and revolution". *Philos Trans R Soc Lond B Biol Sci. 361 (1470): 969–1006.* doi:10.1098/rstb.2006.1842. PMC 1578732. PMID 16754610.
33. *Schopf, J. (1994).* "Disparate rates, differing fates: tempo and mode of evolution changed from the Precambrian to the Phanerozoic". *PNAS. 91 (15): 6735–6742.* Bibcode*:1994PNAS...91.6735S.* doi:10.1073/pnas.91.15.6735. PMC 44277. PMID 8041691.
34. *Stanley, S. (May 1973).* "An Ecological Theory for the Sudden Origin of Multicellular Life in the Late Precambrian". *PNAS. 70 (5): 1486–1489.* Bibcode*:1973PNAS...70.1486S.* doi:10.1073/pnas.70.5.1486. PMC 433525. PMID 16592084.
35. *DeLong, E.; Pace, N. (2001).* "Environmental diversity of bacteria and archaea" (PDF). *Syst Biol. 50 (4): 470–8.* CiteSeerX 10.1.1.321.8828. doi:10.1080/106351501750435040. PMID 12116647.
36. *Schmidt, A.; Ragazzi, E.; Coppellotti, O.; Roghi, G. (2006). "A microworld in Triassic amber". Nature. 444 (7121): 835.* Bibcode*:2006Natur.444..835S.* doi:10.1038/444835a. PMID 17167469.
37. *Schirber, Michael (27 July 2014).* "Microbe's Innovation May Have Started Largest Extinction Event on Earth". *Space.com. Astrobiology Magazine. That spike in nickel allowed methanogens to take off.*
38. *Wolska, K. (2003). "Horizontal DNA transfer between bacteria in the environment". Acta Microbiol Pol. 52 (3): 233–243.* PMID 14743976.
39. *Enright, M.; Robinson, D.; Randle, G.; Feil, E.; Grundmann, H.; Spratt, B. (May 2002).* "The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA)". *Proc Natl*

Acad Sci USA. 99 (11): 7687–7692. Bibcode*:2002PNAS...99.7687E.* doi*:*10.1073/pnas.122108599*.* PMC 124322*.* PMID 12032344*.*

40. "Deep sea microorganisms and the origin of the eukaryotic cell" (PDF). Retrieved 24 October 2017*.*
41. *Yamaguchi, Masashi; et al. (1 December 2012). "Prokaryote or eukaryote? A unique microorganism from the deep sea". Journal of Electron Microscopy. 61 (6): 423–431.* doi*:*10.1093/jmicro/dfs062*.* PMID 23024290*.*
42. *Woese, C.; Kandler, O.; Wheelis, M. (1990).* "Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya"*. Proc Natl Acad Sci USA. 87 (12): 4576–9.* Bibcode*:1990PNAS...87.4576W.* doi*:*10.1073/pnas.87.12.4576*.* PMC 54159*.* PMID 211 2744*.*
43. *De Rosa, M.; Gambacorta, A.; Gliozzi, A. (1 March 1986).* "Structure, biosynthesis, and physicochemical properties of archaebacterial lipids"*. Microbiol. Rev. 50 (1): 70–80.* PMC 373054*.* PMID 3083222*.*
44. *Robertson, C.; Harris, J.; Spear, J.; Pace, N. (2005). "Phylogenetic diversity and ecology of environmental Archaea". Curr Opin Microbiol. 8 (6): 638–42.* doi*:*10.1016/j.mib.2005.10.003*.* PMID 16236543*.*
45. *Karner, M.B.; DeLong, E.F.; Karl, D.M. (2001). "Archaeal dominance in the mesopelagic zone of the Pacific Ocean". Nature. 409 (6819): 507–10.* Bibcode*:2001Natur.409..507K.* doi*:*10.1038/35054051*.* PMID 11206545*.*
46. *Sinninghe Damsté, J.S.; Rijpstra, W.I.; Hopmans, E.C.; Prahl, F.G.; Wakeham, S.G.; Schouten, S. (June 2002).* "Distribution of Membrane Lipids of Planktonic Crenarchaeota in the Arabian Sea"*. Appl. Environ. Microbiol. 68 (6): 2997–3002.* doi*:*10.1128/AEM.68.6.2997-3002.2002*.* PMC 123986*.* PMID 12039760*.*
47. *Leininger, S.; Urich, T.; Schloter, M.; Schwark, L.; Qi, J.; Nicol, G. W.;* Prosser, J. I.*; Schuster, S. C.; Schleper, C. (2006). "Archaea predominate among ammonia-oxidizing prokaryotes in soils".* Nature*. 442 (7104): 806–809.* Bibcode*:2006Natur.442..806L.* doi*:*10.1038/nature04983*.* PMID 16915287*.*
48. *Gold, T. (1992).* "The deep, hot biosphere"*. Proc. Natl. Acad. Sci. U.S.A. 89 (13): 6045–9.* Bibcode*:1992PNAS...89.6045G.* doi*:*10.1073/pnas.89.13.6045*.* PMC 49434*.* PMID 163 1089*.*
49. *Whitman, W.; Coleman, D.; Wiebe, W. (1998).* "Prokaryotes: The unseen majority"*. PNAS. 95 (12): 6578–83.* Bibcode*:1998 PNAS...95.6578W.* doi*:*10.1073/pnas.95.12.6578*.* PMC 33863*.* PMID 9618454*.*
50. *Staff (2 May 2016).* "Researchers find that Earth may be home to 1 trillion species"*.* National Science Foundation*.* Retrieved 6 May 2016*.*
51. *Schulz, H.; Jorgensen, B. (2001). "Big bacteria". Annu Rev Microbiol. 55: 105–37.* doi*:*10.1146/annurev.micro.55.1.105*.* PMID 11544351*.*
52. Shapiro, J.A. *(1998).* "Thinking about bacterial populations as multicellular organisms" (PDF)*. Annu. Rev. Microbiol. 52: 81–104.* doi*:*10.1146/annurev.micro.52.1.81*.* PMID 9891794*. Archived from* the original (PDF) *on 17 July 2011.*

53. *Muñoz-Dorado, J.; Marcos-Torres, F. J.; García-Bravo, E.; Moraleda-Muñoz, A.; Pérez, J. (2016).* "Myxobacteria: Moving, Killing, Feeding, and Surviving Together". *Frontiers in Microbiology. 7: 781.* doi:10.3389/fmicb.2016.00781. PMC 4880591. PMID 27303375.
54. *Johnsbor, O.; Eldholm, V.; Håvarstein, L.S. (December 2007). "Natural genetic transformation: prevalence, mechanisms and function". Res. Microbiol. 158 (10): 767–78.* doi:10.1016/j.resmic.2007.09.004. PMID 17997281.
55. *Eagon, R. (1962).* "Pseudomonas Natriegens, a Marine Bacterium With a Generation Time of Less Than 10 Minutes". *J Bacteriol. 83 (4): 736–7.* PMC 279347. PMID 13888946.
56. Eukaryota: More on Morphology. (Retrieved 10 October 2006)
57. *Dyall, S.; Brown, M.; Johnson, P. (2004). "Ancient invasions: from endosymbionts to organelles". Science. 304(5668): 253–7.* Bibcode:*2004 Sci...304..253D.* doi:10.1126/science.1094884. PMID 15073369. Coenocyte.
58. *Bernstein, H.; Bernstein, C.; Michod, R.E. (2012).* "Chapter 1". *In Kimura, Sakura; Shimizu, Sora. DNA repair as the primary adaptive function of sex in bacteria and eukaryotes. DNA Repair: New Research. Nova Sci. Publ. pp. 1–49.* ISBN 978-1-62100-808-8.
59. Cavalier-Smith T *(1 December 1993).* "Kingdom protozoa and its 18 phyla". *Microbiol. Rev. 57 (4): 953–994.* PMC 372943. PMID 8302;218.
60. *Corliss JO (1992). "Should there be a separate code of nomenclature for the protists?". BioSystems. 28 (1–3): 1–14.* doi:10.1016/0303-2647(92)90003-H. PMID 1292654.
61. *Devreotes P (1989). "Dictyostelium discoideum: a model system for cell-cell interactions in development". Science. 245 (4922): 1054–8.* Bibcode:*1989Sci...245.1054D.* doi:10.1126/science.2672337. PMID 2672337.
62. *Slapeta, J; Moreira, D; López-García, P. (2005).* "The extent of protist diversity: insights from molecular ecology of freshwater eukaryotes". *Proc. Biol. Sci. 272 (1576): 2073–2081.* doi:10.1098/rspb.2005.3195. PMC 1559898. PMID 16191619.
63. *Moreira, D.; López-García, P. (2002).* "The molecular ecology of microbial eukaryotes unveils a hidden world"(PDF). *Trends Microbiol. 10 (1): 31–8.* doi:10.1016/S0966-842X(01)02257-0. PMID 11755083.
64. *Kumamoto, C.A.; Vinces, M.D. (2005). "Contributions of hyphae and hypha-co-regulated genes to Candida albicans virulence". Cell. Microbiol. 7 (11): 1546–1554.* doi:10.1111/j.1462-5822.2005.00616.x. PMID 16207242.
65. *Thomas, David C. (2002). Seaweeds. London: Natural History Museum.* ISBN 978-0-565-09175-0.
66. *Szewzyk, U; Szewzyk, R; Stenström, T. (1994).* "Thermophilic, anaerobic bacteria isolated from a deep borehole in granite in Sweden". *PNAS. 91 (5): 1810–3.* Bibcode:*1994PNAS...91.1810S.* doi:10.1073/pnas.91.5.1810. PMC 43253. PMID 1160 7462.
67. *Horneck, G. (1981). "Survival of microorganisms in space: a review". Adv Space Res. 1 (14): 39–48.* doi:10.1016/0273-1177(81)90241-6. PMID 11541716.
68. *Rousk, Johannes; Bengtson, Per (2014).* "Microbial regulation of global biogeochemical cycles". *Frontiers in Microbiology. 5 (2): 210–25.* doi:10.3389/fmicb.2014.00103. PMC 3954078. PMID 3954078.
69. *Filloux, A.A.M., ed. (2012). Bacterial Regulatory Networks. Caister Academic Press.* ISBN 978-1-908230-03-4.

70. *Gross, R.; Beier, D., eds. (2012). Two-Component Systems in Bacteria. Caister Academic Press.* ISBN 978-1-908230-08-9.
71. *Requena, J.M., ed. (2012). Stress Response in Microbiology. Caister Academic Press.* ISBN 978-1-908230-04-1.
72. Strain 121, a hyperthermophilic archaea, has been shown to reproduce at 121 °C (250 °F), and survive at 130 °C (266 °F).[1]
73. Some Psychrophilic bacteria can grow at −17 °C (1 °F)),[2] and can survive near absolute zero)."Earth microbes on the Moon". *Archived from* the original *on 23 March 2010*. Retrieved 20 July 2009.
74. Dyall-Smith, Mike, HALOARCHAEA, University of Melbourne. See also Haloarchaea.
75. Bacillus alcalophilus can grow at up to pH 11.5
76. Picrophilus can grow at pH −0.06.[3]
77. The piezophilic bacteria Halomonas salaria requires a pressure of 1,000 atm; nanobes, a speculative organism, have been reportedly found in the earth's crust at 2,000 atm.[4]
78. *Anderson, A. W.; Nordan, H. C.; Cain, R. F.; Parrish, G.; Duggan, D. (1956). "Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation". Food Technol. 10 (1): 575–577.*
79. https://en.wikipedia.org/wiki/Microorganism
80. *Faust, Karoline; Raes, Jeroen (16 July 2012). "Microbial interactions: from networks to models". Nature Reviews. Microbiology. 10 (8): 538–550.* doi:10.1038/nrmicro2832. PMID 22796884.
81. *Sheela., Srivastava (2003). Understanding bacteria. Srivastava, P. S. (Prem S.). Dordrecht: Kluwer Academic Publishers.* ISBN 978-1402016332. OCLC 53231924.
82. *López-García, Purificación; Eme, Laura; Moreira, David (December 2017).* "Symbiosis in eukaryotic evolution". *Journal of Theoretical Biology. The origin of mitosing cells: 50th anniversary of a classic paper by Lynn Sagan (Margulis). 434 (Supplement C): 20–33.* doi:10.1016/j.jtbi.2017.02.031. PMC 5638015. PMID 28254477.
83. *García, Cristina; Rendueles, Manuel; Díaz, Mario (September 2017). "Synbiotic Fermentation for the Co-Production of Lactic and Lactobionic Acids from Residual Dairy Whey". Biotechnology Progress. 33 (5): 1250–1256.* doi:10.1002/btpr.2507. PMID 28556559.
84. *I., Krasner, Robert (2010). The microbial challenge : science, disease, and public health (2nd ed.). Sudbury, Mass.: Jones and Bartlett Publishers.* ISBN 9780763756895. OCLC 317664342.
85. https://www.onlinebiologynotes.com/microbial-ecology-and-role-of-microorganism-in-ecosystem

Unit 2 Characteristics of Microorganisms

Unit Structure

2.0 Learning Objectives

After studying this unit you would be able to:

- Explain what are microorganisms and why these are taken up as a unit of study
- Identify the various types of microorganisms
- Explain the Classification of Protist, Virus, Prokaryotic and Eukaryotic cells
- Describe the Structure, Nucleic acids and Replication of Protist, Virus, Prokaryotic and Eukaryotic cells
- Explain the Recombinant DNA, Genetic Engineering of Protist, Virus, Prokaryotic and Eukaryotic cells

2.1 Introduction

In unit 1 you have studied about the general characteristics of microorganisms and the environment in which they live. In this unit you will study about the classification, structure, nucleic acids and replication, recombinant DNA and genetic engineering of microorganisms. Microorganisms are very broadly dispersed. They are found approximately in all places in nature. In common, the circumstances for the growth and multiplication (food, temperature, moisture etc.) are analogous to humans being. Therefore, they are mainly in abundance at places where public exist.

Common kingdoms are animals, plants, bacteria and fungus. In kingdom protista unlike organisms are found. These are eukaryotes, unicellular or multicellular and may live in water, moist places or even inside human body.

Prokaryotes (Pro-primitive, Karyon-nucleus) are the most primordial cells as according to morphology. Examples of prokaryotes are bacteria and blue green algae.

Eukaryotes (Eu-new, Karyon-nucleus) are more complex than prokaryotes. Eukaryotes have membrane bound nucleus and other cell organelles. Examples of eukaryotes are bacteria and archea.

2.3 Protists

Protists are eukaryotic organisms. They contain a nucleus in their cells. They are different from animals, plants or fungus. They do not make a natural group, or clade, as they exclude certain eukaryotes. They frequently grouped mutually similar to algae or invertebrates. According to the five-kingdom system of classification given by Robert Whittaker (1969), the protists are under kingdom Protista. These are unicellular or unicellular-colonial organisms which do not have tissues".

Protists have simple levels of organization, but not essentially have familiar characteristics. When used, the term "protists" is now considered to mean a paraphyletic Protists is a group of organisms which are alike in appearance but of dissimilar biological groups. These organisms are eukaryotes and have dissimilar life cycles, trophic levels, modes of locomotion and cellular structures.

According to the classification system given by Lynn Margulis, protists are microscopic organisms, Large multicellular eukaryotes, as kelp, red algae and slime molds kept under Protoctista. Some others scientists use the term protist for both microbial eukaryotes and macroscopic organisms that do not fit into the other traditional kingdoms.

2.3.1 Subdivisions of Protists

Ernst Haeckel (1866) firstly used the term protista. Protists were usually subdivided into numerous groups based on similarities to the "higher" kingdoms. These groups are as follows:

Protozoa: These are unicellular organisms. These are sometimes heterotrophs, and sometimes parasitic. On the basis of characteristics these are further sub divided. For example, as based on motility, the (flagellated) Flagellata, the (ciliated) Ciliophora, the (phagocytic) amoeba, and the (spore-forming) Sporozoa.

Protophyta: These organisms are autotrophs. Generally unicellular algae are comes under this subdivision.

Molds: These organisms are saprophytes. Examples of these "fungus-like" organisms are slime molds and water molds

Animalcules and the Infusoria groups were firstly classified as microscopic organisms. The word Protozoa was introduced by Georg August Goldfuss (1818) for ciliates and corals. Carl von Siebold (1848) included only animal-like unicellular organisms in this group, such as foraminifera and amoebae.

The formal taxonomic category Protoctista was firstly anticipated by John Hogg (in the early 1860s). He said these organisms as primitive unicellular forms of both plants and animals. He defined the Protoctista as a "fourth kingdom of nature", in adding together to the traditional kingdoms of plants, animals and minerals. Ernst Haeckel (1866) removed Mineral from taxonomy. He explained plants, animals, and the protists (Protista) as a “kingdom of primitive forms”.

Herbert Copeland (1938) said that Protista term given by Ernst Haeckel included anucleated microbes such as bacteria. He also said that it is different from the term "Protoctista"

(literally meaning "first established beings"). In compare to this, Copes land's term included nucleated eukaryotes such as diatoms, green algae and fungi.

On the basis of this classification Whittaker define Fungi, Animalia, Plantae and Protista as the four kingdoms of life. Later on prokaryotes put into the separate kingdom of Monera, and protists as a group of eukaryotic microorganisms.[2]These five kingdoms were acknowledged up to 20th century. In the late 20th century, molecular phylogenetics was developed when it became clear that neither protists nor monera were single groups of linked organisms (they were not monophyletic groups).

2.3.2 Metabolism in Protists

Mode of nutrition can differ as according to the type of protist. Most eukaryotic algae are autotrophic, but in some groups the pigments are absent. Other protists are heterotrophic, and might have phagotrophy, osmotrophy, saprotrophy or parasitism type of mode of nutrition. Some are mixotrophic. Some protists that do not have/lost chloroplasts/mitochondria have endosymbiontic relationship with other bacteria/algae. For example, chloroplast is absent in Paramecium bursaria and Paulinella, are in endosymbiontic relationship with green alga (Zoochlorella) and cyano-bacterium respectively.

Similarly, mitochondria is absent in a protist, Mixotricha paradoxa uses endosymbiontic bacteria as mitochondria and ectosymbiontic hair-like bacteria (*Treponema spirochetes*) for locomotion.

Numerous protists are flagellate. They do filter feeding when come across prey. Some protists engulf bacteria and other food particles, by extending their cell membrane around them, form a food vacuole and digest them within by phagocytosis.

Mode of Nutrition in Protists

Type	Source of energy	Source of carbon	Examples
Photoautotrophs	Sunlight	Organic compounds or carbon fixation	Most algae
Chemoheterotrophs	Organic compounds	Organic compounds	Apicomplexa, Trypanosomes or Amoebae

2.3.3 Reproduction in Protists

Sexual and asexual, both types of reproduction are found in protists. Some reproduce sexually using gametes, whereas others reproduce asexually by binary fission.

The life cycle of Plasmodium falciparum, have exceptionally the characteristics of various organisms. Some of which reproduces sexually and others asexually.

It is considered that the eukaryotes evolve more than 1.5 billion years past. They were likely protists. Although sexual reproduction is common among existing eukaryotes, it seemed not likely until in recent times, that sex could be a primal and basic attribute of eukaryotes. The reason of it was that certain pathogenic protists whose associates split off early from the eukaryotic family tree were deficient in sex. Though, abundant of the protists are now recognized to be competent for meiosis and so sexual reproduction.

Self-Assessment 1

1. Write a short note on Protists.

   _____
   _____
   _____
   _____
   _____
   _____

2. Who firstly used the term Protista?

   _____
   _____
   _____
   _____
   _____

3. Write down the names of numerous groups of Protists based on similarities to the "higher" kingdoms.

   _____
   _____
   _____
   _____
   _____

4. Write down a short note on Protozoa.

5. Who introduced the word Protozoa in 1818 for ciliates and corals?

6. Explain in short the methods of metabolisms in Protists.

7. Who defined Fungi, Animalia, Plantae and Protista as the four kingdoms of life?

8. Which types of reproduction are found in Protists?

2.4 Virus

Virus is a small infectious agent. It replicates only inside the living cells of an organism. Viruses can infect all kinds of organisms, from animals and plants to microorganisms, including bacteria and archaea. Vīrus is a Latin word which meaning is to poison.The term virion (1959) is used for a single viral particle that is released from the cell and has capacity to infect other cells.

Ivanovsky (1892) described a non-bacterial pathogen infecting tobacco plants. Martinus Beijerinck (1898) discovered tobacco mosaic virus. Till the date about 5,000 species of virus have been described in detail, although there are millions of types.Viruses are found in more or less in every ecosystem on Earth. These are the most numerous type of biological creature. The study of viruses is known as virology.

Outside an infected cell, viruses are present in the form of independent particles, known as virions. Virus consists of: (i) the genetic material as long molecules of DNA or RNA that encode the structure of the proteins by which the virus acts; (ii) a protein coat known as capsid, which surrounds and protects the genetic material; and in some cases (iii) an outside envelope of lipids.

2.4.1 Classification of Virus

Classification seeks to describe the diversity of viruses by naming and grouping them on the basis of similarities. André Lwoff, Robert Horne, and Paul Tournier (1962) firstly described the classification of virus, based on the Linnaean hierarchical system. This classification was based on phylum, class, order, family, genus, and species. Viruses were classified as according to their common properties and the type of nucleic acid forming their genomes. The International Committee on Taxonomy of Viruses (ICTV) was created in 1966.

ICTV never fully accepted the classification given by Lwoff, Horne and Tournier because small genome size viruses and their high rate of mutation makes it difficult to verify their origin. The classification given by Baltimore is used to enhance the more conventional hierarchy.

ICTV classification

The International Committee on Taxonomy of Viruses (ICTV) developed the existing classification system and prepared guiding principles to sustain family uniformity. A universal system for classification of viruses was established. Only a small part of the total diversity of viruses has been studied.

The general taxonomic structure of taxon used is as follows:

Phylum -Viricota

Subphylum -Viricotina
Class -Viricetes
Order -Virales
Suborder -Virineae
Family -Viridae
Subfamily -Virinae
Genus -Virus
Subgenus -Virus
Species

As of 2018, 14 orders, 143 families, 64 subfamilies, 846 genera, and 4,958 species of viruses have been defined by the ICTV. The orders are the Caudovirales, Herpesvirales, Ligamenvirales, Mononegavirales, Nidovirales, Orterviral es, Picornavirales, Bunyavirales, Tymovirales, Muvirales, Serpentovirales, Jingchuvirales, Goujianvirales, and Articulavirales.

Baltimore classification

David Baltimore designed the Baltimore classification system of viruses. The ICTV classification system in combination with the Baltimore classification system is used in modern virus classification.

The Baltimore classification of viruses is based on the mechanism of mRNA production. Viruses have to produce mRNAs from their genomes to generate proteins and for their replication. But in each virus family its mechanism is different. Viral genomes may be single-stranded (ss) or double-stranded (ds), RNA or DNA, and may or may not use reverse transcriptase (RT). In addition, ss RNA viruses may be either sense (+) or antisense (−). Viruses have seven groups as according to this classification:

- dsDNA viruses : Adenoviruses, Herpes viruses, Poxviruses
- ssDNA viruses :+ strand or "sense" DNA e.g. Parvo viruses
- dsRNA viruses : Reoviruses
- (+)ssRNA viruses :+ strand or sense RNA, e.g. Picorna viruses, Toga viruses
- (−)ssRNA viruses :− strand or antisense RNA, e.g. Orthomyxo viruses, Rhabdo viruses

- ssRNA-RT viruses :+ strand or sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- dsDNA-RT viruses: DNA with RNA intermediate in life-cycle, e.g. Hepadna viruses

For example, the chicken pox virus, Varicella zoster (VZV), belongs to the order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae, and genus Varicellovirus. VZV is included inthe Group I of the Baltimore Classification because it is a ds DNA virus which does not use reverse transcriptase.

The entire situate of viruses in an organism or habitat is called the virome; for example, all human viruses comprise the human virome.

The origin of viruses in the evolution of life on earth is uncertain. A number of viruses might have evolved from plasmids, pieces of DNA that can move between cells, whereas others might have evolved from bacteria. In evolution, viruses are significant example of horizontal gene transfer, which increases genetic diversity as in sexual reproduction.

Viruses has genetic material, they have reproduction and evolve through natural selection. Due to these characteristics, these are considered to be living by some scientists. But the basic characteristic, the cell structure, to be counted as living is lack. Because of the presence of some characteristics and lack of others, viruses are known as "organisms at the edge of life", and as replicators.

2.4.2 Structure of Virus

The virus particles may be of different shapes from simple helical to icosahedra known as morphologies. viruses are much smaller than bacteria. Virions of most of the species of viruses are too small to be seen with an optical microscope. They can be seen with the help of electron microscopes. These may be about one hundredth the size of most of the bacteria.

Most of the viruses encompass a diameter between 20 and 300 nanometers. Some filoviruses have a total length of up to 1400 nm and diameter only about 80 nm. To amplify the distinguish between viruses and the background, electron-dense "stains" are used. These stains are the solutions of salts of heavy metals, like tungsten. They disperse the electrons from region enclosed with the stain. When virions are coated with stain

(positive staining), excellent features are hidden. Negative staining overcomes this trouble by staining the background only.

A whole virus particle is known as virion. It contains nucleic acid enclosed by a protective coat of protein known as capsid. These are produced from indistinguishable protein subunits known as capsomeres. Viruses have an outer covering known as envelop, made up of lipid and derived from the cell membrane of the host. The capsid is made up of proteins encoded by the viral genome. Its shape is the basis for morphological difference. Generally in the presence of the virus genome virally-coded protein subunits self-assembled to form a capsid .

Complex viruses code for proteins that help in the building of their capsid. Proteins related with nucleic acid are known as nucleoproteins. The alliance of viral capsid proteins with viral nucleic acid is known as nucleocapsid. The capsid and whole virus structure can be mechanically (physically) examined through atomic force microscopy. Commonly, there are four main morphological virus types:

1) Helical: These viruses are made up of a single type of capsomere mound around a central axis and form a helical structure. It may have a central cavity or tube. It forms rod-shaped or filamentous virions which may be short and highly rigid, or long and very flexible. The genetic material (typically single-stranded RNA, but ssDNA in some cases) is bound into the protein helix by exchanges between the negatively charged nucleic acid and positive charges on the protein. The length of a helical capsid is linked to the length of the nucleic acid enclosed within it, and the diameter is dependent on the size and arrangement of capsomeres. The example of a helical virus is Tobacco mosaic virus.

2) Icosahedral: The majority of animal viruses are icosahedral or near-spherical with chiral icosahedral symmetry. A normal icosahedron is the best manner of forming a closed shell from alike sub-units. For every triangular face the minimum essential number of alike capsomeres is 3, which gives 60 for the icosahedron. Many viruses, like rotavirus, have more than 60 capsomers. They look like spherical but they maintain this symmetry. The capsomeres at the tips are enclosed by five other capsomeres and are known as pentons. Capsomeres on the triangular faces are enclosed by six others and are called hexons. Hexons are in core flat and pentons, form the 12 vertices, are curved. The

same protein may be the subunit of both the pentamers and hexamers or they may be of dissimilar proteins.

3) Prolate: This is an icosahedron extended along the fivefold axis and is a general arrangement of the heads of bacteriophages. This structure is composed of a cylinder with a cap at either end.

4) Envelope: Some species of virus enclose themselves in an altered form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes like a nuclear membrane or endoplasmic reticulum. Therefore they get an outer lipid bilayer known as a viral envelope. This membrane is covered with proteins coded for the viral genome and host genome; the lipid membrane and any carbohydrates present derive absolutely from the host. The influenza virus and HIV use this approach. The majority of enveloped viruses are dependent on the envelope for their contagion.

5) Complex: These viruses have a capsid that is neither entirely helical nor wholly icosahedral. These may have extra structures such as protein tails or a composite outer wall. Some bacteriophages, such as Enterobacteria phage T4, have a composite structure made up of an icosahedral head bound to a helical tail. Tail may have a hexagonal base plate with extending protein tail fibres. This tail structure acts like a molecular syringe. It affixes to the bacterial host and then inject viral genome into the cell.

2.4.3 Genome of Virus

Genomic diversity amongst viruses	
Property	Parameters
Nucleic acid	DNA RNA Both DNA and RNA (at different stages in the life cycle)
Shape	Linear Circular Segmented
Strandedness	Single-stranded Double-stranded

	Double-stranded with regions of single-strandedness
Sense	Positive sense (+) Negative sense (−) Ambisense (+/−)

An huge diversity of genomic structures can be seen amongst viral species. Viruses have various structural genome in comparison to plants, animals, archaea, and bacteria. Millions of various types of viruses are present, though we have details of only about 5,000 types. The NCBI Virus genome database (2015) has more than 75,000 total genome sequences,[90] but there are doubtlessly numerous other to be revealed.

A virus has either a DNA or an RNA genome and is called a DNA virus or an RNA virus, correspondingly. Enormously viruses have RNA genomes. Plant viruses have a tendency to have single-stranded RNA genomes and bacteriophages double-stranded DNA genomes.

Viral genomes are circular, as in polyomaviruses, or linear, as in adenoviruses. The type of nucleic acid is unrelated to the shape of the genome. Among RNA viruses and certain DNA viruses, the genome is frequently separated into parts, so it is known as segmented. For RNA viruses, each segment usually codes for only one protein. They are generally found jointly in one capsid.

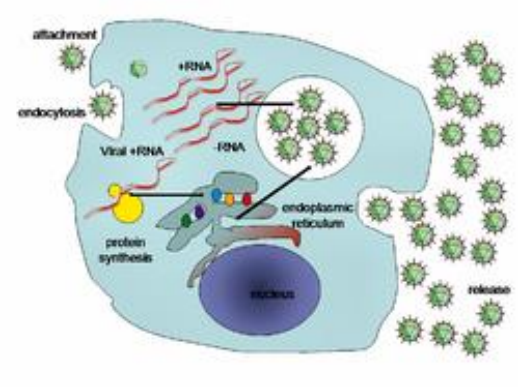
A viral genome,regardless of nucleic acid type, is approximately all the time either single-stranded or double-stranded. Single-stranded genomes contain an unpaired nucleic acid. Double-stranded genomes contain two complementary paired nucleic acids. The virus particles belong to the Hepadnaviridae, have a genome that is partly double-stranded and partly single-stranded.

Genome size: Genome size varies significantly among species. The smallest viral genomes—the ssDNA circoviruses, code for only two proteins and have a genome size of only two kilobases. Pandoraviruses have genome sizes of approximately two megabases which code for about 2500 proteins.

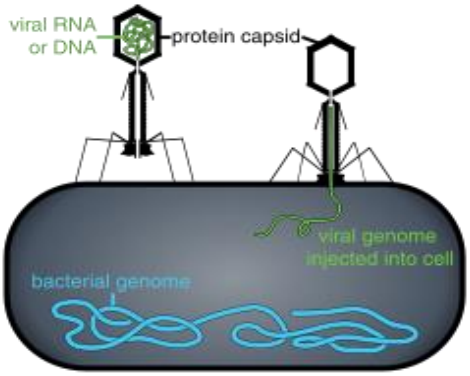
Commonly, RNA viruses have smaller genome sizes than DNA viruses. On the other hand DNA viruses, except single-stranded, usually have larger genomes because of the high precision of the replication enzymes.

2.4.4 Replication Cycle in Virus

Being acellular, viruses do not have cell division. In its place, they use the mechanism and metabolism of a host cell to reproduce, and they accumulate in the cell.



A typical virus replication cycle



Some bacteriophages inject their genomes **into bacterial cells (not to scale)**

The life cycle of viruses vary significantly between species but they have six basic stages in the life cycle of viruses.

Attachment is a particular binding between viral capsid proteins and precise receptors on the cellular surface of the host.

Penetration is the next step. Virions penetrate the host cell all the way through receptor-mediated endocytosis or membrane fusion. This is known as viral entry. The infection of plant and fungal cells is dissimilar from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi of chitin, therefore the majority of viruses can get within these cells only after damage to the cell wall. Almost all plant viruses for example Tobacco mosaic virus can also travel straight from cell to cell, in the form of single-stranded nucleoprotein complexes, all the way through pores known as plasmodesmata.

Bacteria also have tough cell walls that a virus has to break to infect the cell. The cell wall of bacterial is greatly thinner than plant cell walls because of the smaller size. Some viruses insert their genome into the bacterial cell across the cell wall, though the viral capsid remains outer.

Uncoating is the next step. In this process the viral capsid is detached. This may be by degradation by viral enzymes or host enzymes or by simple dissociation. It results in the release of the viral genomic nucleic acid.

Replication of viruses includes principally multiplication of the genome. Replication involves synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, and potential assemblage of viral proteins. Viral genome replication is intervened by early or regulatory protein expression.

Assembly, some modification of the proteins frequently occurs during structure-intervened self-assembly of the virus particles. In HIV, this modification (occasionally known as maturation) occurs after the release of virus from the host cell.

Release – Viruses can be released from the host cell by lysis. This process results in the killing of the cell by bursting its membrane and cell wall if there: This is a characteristic of numerous bacterial and several animal viruses.

Animal viruses

Viruses are important pathogens of farm and domestic animals. Various diseases like foot-and-mouth disease and bluetongue are caused by viruses.

If domestic animals like cats, dogs, and horses not vaccinated, are prone to severe viral infections. Canine parvovirus is caused by a small DNA virus and infections are frequently critical in pups. Like all invertebrates, the honey bee is prone to various viral infections. The majority of viruses co-exists without risk in their host and cause no signs or symptoms of disease.

Plant viruses

There are many types of plant virus. Plant viruses frequently spread from plant to plant by organisms, called vectors. These are generally insects, but some fungi, nematode worms, and single-celled organisms have been revealed as vectors. Plant viruses cannot communicate a disease to humans and other animals because they can reproduce merely in living plant cells.

Bacteriophage is a widespread and dissimilar group of viruses. These are the most plentiful biological creature in aquatic environments. These viruses are up to ten times more in the oceans than the bacteria, These may be of 250,000,000 bacteriophages per millilitre of seawater.[218] These viruses infect particular bacteria by binding to surface receptor molecules and then penetrate into the cell.

Peppers infected by mild mottle virus

Main article: Plant virus

Archaeal viruses

Some viruses replicate/reproduce within archaea. These are double-stranded DNA viruses with remarkable and sometimes distinctive shapes. These viruses have been studied in most detail in the thermophilic archaea, mainly the orders Sulfolobales and Thermoproteales.

Self-Assessment 2

1. Vīrus is a Latin word what is the meaning of it?

_____

_____

_____

_____

2. Which word is used for a single viral particle?

_____

_____

_____

_____

_____

3. Who described a non-bacterial pathogen infecting tobacco plants?

4. Who discovered tobacco mosaic virus?

5. What are the constituents of the structure of a virus?

6. Which organization developed the existing classification system and prepared guiding principles to sustain family uniformity?

7. Write down the names of the various shapes of virus particles.

_____

_____

8. Why the process of cell division is absent in viruses?

_____

_____

_____

_____

9. Which are the six basic stages in the life cycle of viruses?

_____

_____

_____

_____

2.5 Prokaryotes

A unicellular organism that is deficient of a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle. Prokaryote comes from the Greek προ (pro, 'before') and κάρυον (karyon, 'nut' or 'kernel'). Prokaryotes are divided into two parts, Archaea and Bacteria. The species which have nuclei and organelles are known as Eukaryota.

Roger Stanier and C. B. van Niel (1962) discribed the difference between prokaryotes and eukaryotes in the research paper The concept of a bacterium.

2.5.1 Structure of Prokaryotes

Prokaryotes have a prokaryotic cytoskeleton that is more primordial than that of the eukaryotes. Apart from homologues of actin and tubulin, the helically arranged building-block of flagellum, flagellin, is one of the most important cytoskeletal proteins of bacteria. It gives structural backgrounds of chemotaxis which is the basic cell physiological response of bacteria.

The size of the majority of the prokaryotes is between 1 µm and 10 µm, but may range from 0.2 µm (Mycoplasma genitalium) to 750 µm (Thiomargarita namibiensis).

Structure of Prokaryotic Cell
Flagellum (only in some types of prokaryotes) Long, whip-like projection that supports cellular locomotion used by both gram positive and gram negative organisms.
Cell membrane Encloses the cytoplasm of cell and controls the current of substances in and out of the cell.
Cell wall (except genera Mycoplasma and Thermoplasma) Outer covering of the majority of cells that defends the bacterial cell and provides the shape.
Cytoplasm A gel-like material composed primarily of water that as well have enzymes, salts, cell components, and a variety of organic molecules.
Ribosome Cell organelle accountable for protein synthesis.
Nucleoid Region of the cytoplasm that have the single DNA molecule of the prokaryote
Glycocalyx (only in some types of prokaryotes) A glycoprotein-polysaccharide layer that encases the cell membranes.
Inclusions It restrains the inclusion bodies like ribosomes and bigger masses spread in the cytoplasmic matrix.

2.5.2 Morphology of Prokaryotes

Prokaryotic cells have different shapes. The four basic shapes of bacteria are as follows:

- Cocci – A bacterium that is spherical or ovoid is called a coccus (Plural, cocci). e.g. Streptococcus, Staphylococcus.
- Bacilli – A bacterium with cylindrical shape called rod or a bacillus (Plural, bacilli).
- Spiral bacteria – Some rods twist into spiral shapes and are called spirilla (singular, spirillum).

- Vibrio – comma-shaped

The archaeon Haloquadratum has flat square-shaped cells.

Among archaea, Halobacterium volcanii forms cytoplasmic connection between cells that come into view to be used for transfer of DNA from one cell to another. Another archaeon, Sulfolobus solfataricus, transfers DNA between cells by direct contact.

2.5.3 Reproduction in Prokaryotes

Asexual reproduction is found in bacteria and archaea. It is generally by binary fission. Yet genetic exchange and recombination exist, but this is a form of horizontal gene transfer and is not a replicative process. It simply involves the transference of DNA between two cells, as in bacterial conjugation.

Transfer of DNA: Transfer of DNA between prokaryotic cells takes place in bacteria and archaea, though it has been mostly studied in bacteria. In bacteria, gene transfer takes place by three processes. These process are (1) bacterial virus (bacteriophage)-mediated transduction, (2) plasmid-mediated conjugation, and (3) natural transformation.

Transduction of bacterial genes by bacteriophage appears to reflect an occasional error during intracellular assembly of virus particles, rather than an adaptation of the host bacteria. The transfer of bacterial DNA is organized by the bacteriophage's genes rather than bacterial genes. Conjugation is an adaptation to share out copies of a plasmid from one bacterial host to another. It is well-studied in E. coli. All through this process, a plasmid may assimilate into the host bacterial chromosome, and consequently transmit fraction of the host bacterial DNA to another bacterium. Plasmid mediated transmits of host bacterial DNA (conjugation) also appears to be an unplanned practice rather than a bacterial adaptation.

Self-Assessment 3

1. Write down the name of a unicellular organism that is deficient of a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.

   _____
   _____
   _____
   _____
   _____

2. Who described the differences between prokaryotes and eukaryotes and in which research paper?

3. What is the size of the majority of the prokaryotes?

4. What are the four basic shapes of bacteria?

5. Which type of reproduction is found in bacteria and archaea?

6. Write down the name of three processes include in gene transfer in bacteria.

7. Write down a note on flagellum of prokaryotes?

8. What do you understand by cytoplasm in prokaryotes?

9. Which organelle is the site for protein synthesis?

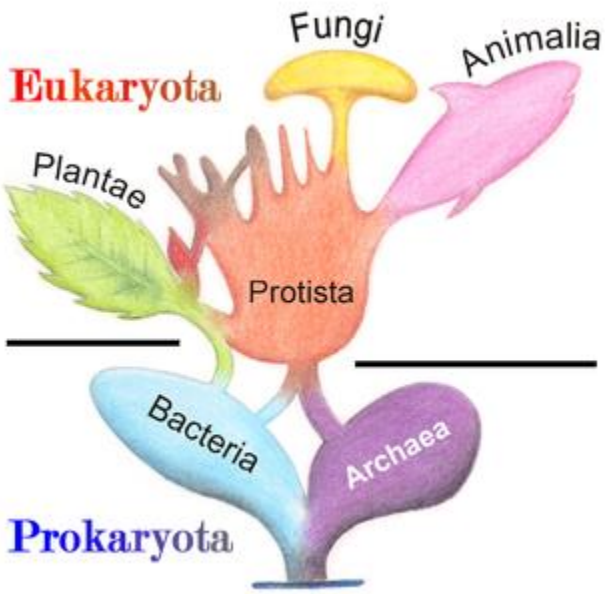
2.6 Eukaryotes

Eukaryotes are organisms that have true nucleus i.e. their cells have a nucleus enclosed or surrounded by membranes. Eukaryota name comes from the Greek εὖ (eu, "well" or "true") and κάρυον (karyon, "nut" or "kernel"). These cells also have other membrane-bound organelles as mitochondria and the Golgi apparatus. Several cells of plants and algae have chloroplasts. Eukaryotes may also be multicellular. These may consist of organisms having many cell types forming diverse types of tissue. The well-known eukaryotes are animals and plants.

The conception of eukaryote has been credited to the French biologist Edouard Chatton (1883–1947). The terms prokaryote and eukaryote were further decisively reinstituted by the Canadian microbiologist Roger Stanier and the Dutch-American microbiologist C. B. van Niel (1962). Chatton (1937) anticipated two terms, called the bacteria prokaryotes and organisms with nuclei in their cells eukaryotes, in his work Titres et Travaux Scientifiques.

2.6.1 Classification of Eukaryotes

In olden days, animals and plants were documented as kingdom by Linnaeus. He put the fungi with plants with a few uncertainties. Later fungi were kept in separate kingdom. A variety of single cell eukaryotes were initially kept with plants or animals. A German biologist Georg A. Goldfuss (1818) mentioned the word protozoa to ciliates. This group was prolonged and took all single-celled eukaryotes and specified an individual kingdom, the Protista, by Ernst Haeckel (1866). The eukaryotes have four kingdoms:



- Kingdom Protista
- Kingdom Plantae

- Kingdom Fungi
- Kingdom Animalia

The classification formed in 2005 by the International Society of Protistologists,[separated the eukaryotes into six apparently monophyletic 'supergroups'. A revised classification in 2012 gave five super groups.

Archaeplastida (or Primoplantae)	Land plants, green algae, red algae, and glaucophytes
SAR supergroup	Stramenopiles (brown algae, diatoms, etc.), Alveolata, and Rhizaria (Foraminifera, Radiolaria, and various other amoeboid protozoa)
Excavata	Various flagellate protozoa
Amoebozoa	Most lobose amoeboids and slime molds
Opisthokonta	Animals, fungi, choanoflagellates, etc.

2.6.2 Cell Features of Eukaryotes

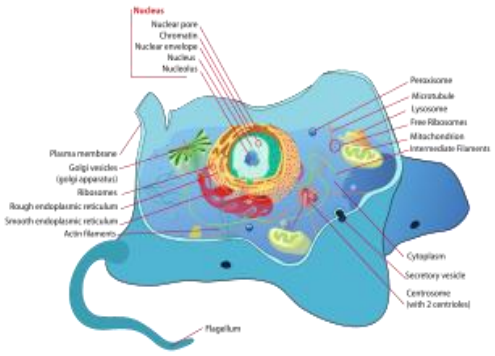
Eukaryotic cells are characteristically bigger than prokaryotes. These cells have a volume of approximately 10,000 times greater than the prokaryotic cell. They have various internal membrane-bound organelles. These cells have cytoskeleton composed of microtubules, microfilaments, and intermediate filaments, which are significant for the organization and shape of the cells. DNA has chromosomes as linear bundles, which are separated by a microtubular spindle for the duration of nuclear division.

Internal membrane

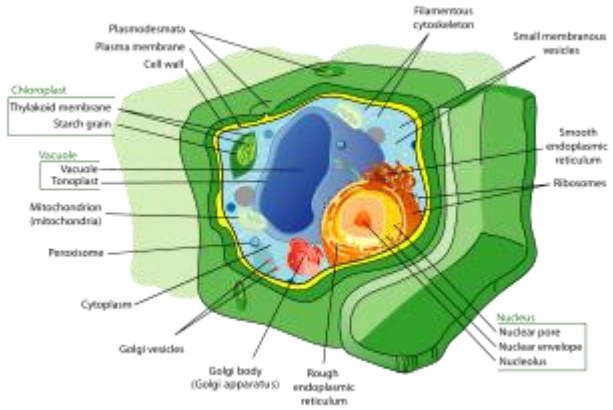
The endomembrane system and its components. Eukaryote cells contain various membrane-bound structures, as a group called as the endomembrane system. Simple compartments, called vesicles and vacuoles, can form by budding off other membranes. Endocytosis is found, a process in which many cells ingest food and other

materials. It is probable that most other membrane-bound organelles are ultimately derived from such vesicles. Some waste products formed by the cell can go away in a vesicle through exocytosis.

The nucleus is enclosed by a double membrane generally named as nuclear membrane or nuclear envelope. It has pores which permit matter to move in and out. Protein transport and maturation is done by endoplasmic reticulum. These are a mixture of tube and sheet like extensions of the nuclear membrane. Ribosomes synthesize proteins, are attached to rough endoplasmic reticulum. It goes into the inner space or lumen. Eukaryotic cells contain golgi apparatus, lysosomes, peroxysomes and a central vacuole.



Structure of a typical animal cell



Structure of a typical plant cell

2.6.3 Different Types of Eukaryotic Cells

There are numerous kinds of eukaryotic cells. However animals and plants are the mainly well-known eukaryotes, and thus provide an excellent starting point for understanding eukaryotic structure. Fungi and several protists have some significant difference.

Animal cell: All animals are devoid of cell walls and chloroplasts. They have smaller vacuoles than plants. Animal cells have a range of shapes because of the devoid of cell wall.

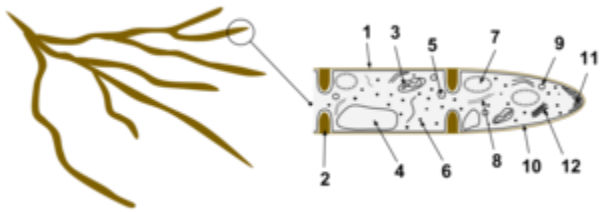
Plant cell: Plant cells are relatively different from eukaryotic cells. Their characteristic features are:

- A large central vacuole surrounded by a membrane known as tonoplast.
- A primary cell wall having cellulose, hemicellulose and pectin.

- Tiny pores are present in the cell wall that connect neighboring cells are known as plasmodesmata.[32] Animals have a different but similar in functions system of gap junctions between neighboring cells.
- Plastids are present, particularly chloroplasts which have chlorophyll. This pigment gives green color to the plants and help in photosynthesis.

Fungi: The cells of fungi are mainly alike to animal cells. Some exceptions are as follows:

- A cell wall have chitin
- Less partition between cells
- Merely the most ancient fungi, chytrids, have flagella.



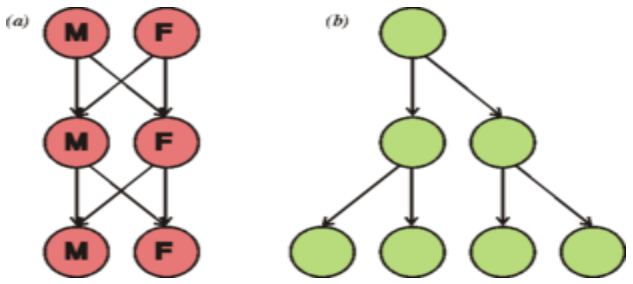
Fungal Hyphae cells: 1 – hyphal wall, 2 – septum, 3 – mitochondrion, 4 – vacuole, 5 – ergosterol crystal, 6 – ribosome, 7 – nucleus, 8 – endoplasmic reticulum, 9 – lipid body, 10 – plasma membrane, 11 – spitzenkörper, 12 – Golgi apparatus

Other eukaryotic cells: Some groups of eukaryotes have unique organelles, such as the cyanelles (unusual chloroplasts) of the glaucophytes, the haptonema of the haptophytes, or the ejectosomes of the cryptomonads. Other structures, such as pseudopodia, are found in various eukaryote groups in different forms, such as the lobose amoebozoans or the reticulose foraminiferans.

2.6.4 Reproduction in Eukaryotes

Asexual and sexual reproduction is present. Mitosis and meiosis types of cell division are found. In asexual reproduction cell division usually takes place by mitosis. In mitosis every daughter nucleus gets one copy of every chromosome. In the majority of eukaryotes sexual reproduction is found. It has both a haploid phase and a diploid phase. In haploid phase, only one copy of every chromosome is present in every cell, whereas in a diploid phase, two copies of every chromosome are present in every cell. Two haploid gametes fuse to form a

zygote in the diploid phase, which may divide by mitosis or go through chromosome reduction by meiosis. Eukaryotes have a lesser surface area to volume ratio than prokaryotes. So they have lesser metabolic rates and longer generation times.



This diagram illustrates the twofold cost of sex. If each individual were to contribute to the same number of offspring (two), (a) the sexual population remains the same size each generation, where the (b) asexual population doubles in size each generation.

Self-Assessment 4

1. Name the organisms that have true nucleus.

_____

_____

_____

_____

2. What is the meaning of true nucleus?

_____

_____

_____

_____

3. Write the name of the well-known eukaryotes.

_____

_____

_____

_____

4. Who mentioned the word protozoa to ciliates?

_____

_____

_____

_____

5. Write the name of the four kingdoms of eukaryotes.

_____

_____

_____

_____

6. Write the name of the six monophyletic 'supergroups' of Eukaryotes.

_____

_____

_____

_____

7. What is endocytosis and exocytosis processes?

_____

_____

_____

_____

8. Why eukaryotes have lesser metabolic rates and longer generation times than prokaryotes?

_____

_____

_____

_____

Summary

In this chapter you have learnt that:

- Protists are eukaryotic organisms. They contain a nucleus in their cells.
- They are different from animals, plants or fungus.
- According to the five-kingdom system of classification given by Robert Whittaker (1969), the protists are under kingdom Protista.

- These are unicellular or unicellular-colonial organisms which do not have tissues"
- Most eukaryotic algae are autotrophic, but in some groups the pigments are absent.
- Other protists are heterotrophic, and might have phagotrophy, osmotrophy, saprotrophy or parasitism type of mode of nutrition.
- Virus is a small infectious agent. It replicates only inside the living cells of an organism.
- The virus particles may be of different shapes from simple helical to icosahedra
- A virus has either a DNA or an RNA genome and is called a DNA virus or an RNA virus, correspondingly.
- Bacteriophage is a widespread and dissimilar group of viruses.
- Prokaryote is a unicellular organism that is deficient of a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.
- The size of the majority of the prokaryotes is between 1 µm and 10 µm.
- Prokaryotic cells have different shapes. The four basic shapes of bacteria are cocci, bacillus, spiral, vibrio.
- Asexual reproduction is found in bacteria and archaea.
- In bacteria, gene transfer takes place by three processes, as (1) bacterial virus (bacteriophage)-mediated transduction, (2) plasmid-mediated conjugation, and (3) natural transformation.
- Eukaryotes are organisms that have true nucleus i.e. their cells have a nucleus enclosed or surrounded by membranes.
- The well-known eukaryotes are animals and plants.
- Eukaryotes has four kingdoms:Kingdom Protista, Plantae, Fungi and Animalia.
- Asexual and sexual reproduction is present in eukaryotes. Mitosis and meiosis types of cell division are found.
- Eukaryotes have a lesser surface area to volume ratio than prokaryotes. So they have lesser metabolic rates and longer generation times.

Terminal Questions

1. What are protists? Explain the subdivisions of protists.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

2. Explain the mode of nutrition in protists.

3. Explain the classification of virus in detail.

4. Explain the structure of virus. What are four main morphological virus types?

5. Write in detail about the genomic diversity amongst viruses.

6. Explain in detail about the six basic stages of the life cycle of viruses.

7. Explain in detail about the structure and morphology of prokaryotes.

8. Write a detailed note on various types of eukaryotic cells.

Answers

Self-Assessment 1

1. Refer to section 2.3
2. Refer to section 2.3.1
3. Refer to section 2.3.1
4. Refer to section 2.3.1
5. Refer to section 2.3.1
6. Refer to section 2.3.2
7. Refer to section 2.3.1

8. Refer to section 2.3.3

Self-Assessment 2

1. Refer to section 2.4
2. Refer to section 2.4
3. Refer to section 2.4
4. Refer to section 2.4
5. Refer to section 2.4
6. Refer to section 2.4.1
7. Refer to section 2.4.2
8. Refer to section 2.4.4
9. Refer to section 2.4.4

Self-Assessment 3

1. Refer to section 2.5
2. Refer to section 2.5
3. Refer to section 2.5.1
4. Refer to section 2.5.2
5. Refer to section 2.5.3
6. Refer to section 2.5.3
7. Refer to section 2.5.1
8. Refer to section 2.5.1
9. Refer to section 2.5.1

Self-Assessment 4

1. Refer to section 2.6
2. Refer to section 2.6
3. Refer to section 2.6
4. Refer to section 2.6.1
5. Refer to section 2.6.1
6. Refer to section 2.6.1
7. Refer to section 2.2.2
8. Refer to section 2.6.4

Terminal Questions

1. Refer to section 2.3 & 2.3.1
2. Refer to section 2.3.2

3. Refer to section 2.4.1
4. Refer to section 2.4.2
5. Refer to section 2.4.3
6. Refer to section 2.4.4
7. Refer to section 2.5.1 & 2.5.2
8. Refer to section 2.6.3

References

10. Whittaker RH (January 1969). "New concepts of kingdoms or organisms. Evolutionary relations are better represented by new classifications than by the traditional two kingdoms". Science. 163 (3863): 150–60. Bibcode:1969Sci...163..150W. CiteSeerX 10.1.1.403.5430. doi:10.1126/science.163.3863.150. PMID 5762760.
11. Whittaker RH (1959). "On the Broad Classification of Organisms". Quarterly Review of Biology. 34 (3): 210–226. doi:10.1086/402733. JSTOR 2816520.
12. "Systematics of the Eukaryota". Retrieved 2009-05-31.
13. Simonite T (November 2005). "Protists push animals aside in rule revamp". Nature. 438 (7064): 8–9. Bibcode:2005Natur.438....8S. doi:10.1038/438008b. PMID 16267517.
14. Harper D, Benton, Michael (2009). Introduction to Paleobiology and the Fossil Record. Wiley-Blackwell. p. 207. ISBN 978-1-4051-4157-4.
15. Margulis L, Chapman MJ (2009-03-19). Kingdoms and Domains: An Illustrated Guide to the Phyla of Life on Earth. Academic Press. ISBN 9780080920146.
16. The Flagellates. Unity, diversity and evolution. Ed.: Barry S. C. Leadbeater and J. C. Green Taylor and Francis, London 2000, p. 3.
17. Goldfuß (1818). "Ueber die Classification der Zoophyten" [On the classification of zoophytes]. Isis, Oder, Encyclopädische Zeitung von Oken (in German). 2 (6): 1008–1019. From p. 1008: "Erste Klasse. Urthiere. Protozoa." (First class. Primordial animals. Protozoa.) [Note: each column of each page of this journal is numbered; there are two columns per page.]

18. Scamardella JM (1999). "Not plants or animals: a brief history of the origin of Kingdoms Protozoa, Protista and Protoctista"(PDF). International Microbiology. 2: 207–221.

19. Siebold (vol. 1); Stannius (vol. 2) (1848). Lehrbuch der vergleichenden Anatomie [Textbook of Comparative Anatomy] (in German). vol. 1: Wirbellose Thiere (Invertebrate animals). Berlin, (Germany): Veit & Co. p. 3. From p. 3: "Erste Hauptgruppe. Protozoa. Thiere, in welchen die verschiedenen Systeme der Organe nicht scharf ausgeschieden sind, und deren unregelmässige Form und einfache Organisation sich auf eine Zelle reduziren lassen." (First principal group. Protozoa. Animals, in which the different systems of organs are not sharply separated, and whose irregular form and simple organization can be reduced to one cell.)

20. Hogg, John (1860). "On the distinctions of a plant and an animal, and on a fourth kingdom of nature". Edinburgh New Philosophical Journal. 2nd series. 12: 216–225. From p. 223: " … I here suggest a fourth or an additional kingdom, under the title of the Primigenal kingdom, … This Primigenal kingdom would comprise all the lower creatures, or the primary organic beings, – 'Protoctista,' – from πρώτος, first, and χτιστά, created beings; … "

21. Rothschild LJ (1989). "Protozoa, Protista, Protoctista: what's in a name?". Journal of the History of Biology. 22 (2): 277–305. doi:10.1007/BF00139515. PMID 11542176.

22. Haeckel, Ernst (1866). Generelle Morphologie der Organismen [The General Morphology of Organisms] (in German). vol. 1. Berlin, (Germany): G. Reimer. pp. 215ff. From p. 215: "VII. Character des Protistenreiches." (VII. Character of the kingdom of Protists.)

23. Copeland HF (1938). "The Kingdoms of Organisms". Quarterly Review of Biology. 13 (4): 383–420. doi:10.1086/394568. JSTOR 2808554.

24. Stechmann A, Cavalier-Smith T (September 2003). "The root of the eukaryote tree pinpointed" (PDF). Current Biology. 13 (17): R665–6. doi:10.1016/S0960-9822(03)00602-X. PMID 12956967.

25. Talman AM, Domarle O, McKenzie FE, Ariey F, Robert V (July 2004). "Gametocytogenesis: the puberty of Plasmodium falciparum". Malaria Journal. 3: 24. doi:10.1186/1475-2875-3-24. PMC 497046. PMID 15253774.

26. Javaux EJ, Knoll AH, Walter MR (July 2001). "Morphological and ecological complexity in early eukaryotic ecosystems". Nature. 412(6842): 66–9. doi:10.1038/35083562. PMID 11452306.

27. Whittaker RH (January 1969). "New concepts of kingdoms or organisms. Evolutionary relations are better represented by new classifications than by the traditional two kingdoms". Science. 163 (3863): 150-60. Bibcode:1969

28. sci...163..150W. CiteSeerX 10.1.1.403.5430. doi:10.1126/science.163.3863.150. PMID 5762760.

29. Whittaker new concepts of kingdoms – Google Scholar". scholar.google.ca. Retrieved 2016-02-28.

30. Whittaker RH (1959). "On the Broad Classification of Organisms". Quarterly Review of Biology. 34 (3): 210–226. doi:10.1086/402733. JSTOR 2816520.

31. "Systematics of the Eukaryota". Retrieved 2009-05-31.

32. Simonite T (November 2005). "Protists push animals aside in rule revamp". Nature. 438 (7064): 8–9. Bibcode:2005Natur.438....8S. doi:10.1038/438008b. PMID 16267517.

33. Harper D, Benton, Michael (2009). Introduction to Paleobiology and the Fossil Record. Wiley-Blackwell. p. 207. ISBN 978-1-4051-4157-4.

34. Margulis L, Chapman MJ (2009-03-19). Kingdoms and Domains: An Illustrated Guide to the Phyla of Life on Earth. Academic Press. ISBN 9780080920146.

35. Koonin EV, Senkevich TG, Dolja VV. The ancient Virus World and evolution of cells. Biology Direct. 2006;1:29. doi:10.1186/1745-6150-1-29. PMID 16984643.

36. Dimmock p. 4

37. Dimmock p. 49

38. Breitbart M. Here a virus, there a virus, everywhere the same virus? Trends in Microbiology. 2005;13(6):278–84. doi:10.1016/j.tim.2005.04.003. PMID 15936660.

39. Lawrence CM, Menon S, Eilers BJ, et al.. Structural and functional studies of archaeal viruses. The Journal of Biological Chemistry. 2009; 284(19):12599–603. doi:10.1074/jbc.R800078200. PMID 19158076.

40. Edwards RA, Rohwer F. Viral metagenomics. Nature Reviews Microbiology. 2005;3(6):504–10. doi:10.1038/nrmicro1163. PMID 15886693.

41. Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brüssow H. Phage as agents of lateral gene transfer. Current Opinion in Microbiology. 2003;6(4):417–24. doi:10.1016/S1369-5274(03)00086-9. PMID 12941415.

42. Rybicki, EP. The classification of organisms at the edge of life, or problems with virus systematics. South African Journal of Science. 1990; 86: 182–86.

43. Are viruses alive? The replicator paradigm sheds decisive light on an old but misguided question. Studies in History and Philosophy of Biological and Biomedical Sciences. 7 March 2016;59:125–34. doi:10.1016/j.shpsc.2016.02.016. PMID 26965225.

44. "virus, n." OED Online. Oxford University Press, March 2015. Web. 23 March 2015.

45. Harper D. The Online Etymology Dictionary. virus; 2011 [Retrieved 19 December 2014].

46. "virulent, adj." OED Online. Oxford University Press, March 2015. Web. 23 March 2015.

47. Casjens S. In: Mahy BWJ and Van Regenmortel MHV. Desk Encyclopedia of General Virology. Boston: Academic Press; 2010. ISBN 978-0-12-375146-1. p. 167.

48. Temin HM, Baltimore D. RNA-directed DNA synthesis and RNA tumor viruses. Advances in Virus Research. 1972;17:129–86. doi:10.1016/S0065-3527(08)60749-6. PMID 4348509.

49. Sanjuán R, Nebot MR, Chirico N, Mansky LM, Belshaw R. Viral mutation rates. Journal of Virology. 2010; 84(19):9733–48. doi:10.1128/JVI.00694-10. PMID 20660197.

50. Rossmann MG, Mesyanzhinov VV, Arisaka F, Leiman PG. The bacteriophage T4 DNA injection machine. Current Opinion in Structural Biology. 2004;14(2):171–80. doi:10.1016/j.sbi.2004.02.001. PMID 15093831.

51. Prangishvili D, Forterre P, Garrett RA. Viruses of the Archaea: a unifying view. Nature Reviews Microbiology. 2006;4(11):837–48. doi:10.1038/nrmicro1527. PMID 17041631.

52. ncbi.nlm.nih.gov. NCBI Viral Genome database[Retrieved 15 January 2017].

53. Pennisi E. Going Viral: Exploring the Role Of Viruses in Our Bodies. Science. 2011;331(6024):1513. doi:10.1126/science.331.6024.1513. PMID 21436418. Bibc ode: 2011Sci...331.1513P.

54. Redefining the invertebrate RNA virosphere. Nature. 2016;540(7634):539–43. doi:10.1038/nature20167. PMID 27880757. Bibcode: 2016Natur.540.539S.Coll ier pp. 96–99

55. Sequences from ancestral single-stranded DNA viruses in vertebrate genomes: the parvoviridae and circoviridae are more than 40 to 50 million years old. Journal of Virology. 2010;84(23):12458–62. doi:10.1128/JVI.01789-10. PMID 20861255.

56. Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. Science. 2013;341(6143):281–86. doi:10.1126/science.1239181. PMID 23869018. Bibcode: 2013Sci...341.281P

57. Pressing J, Reanney DC. Divided genomes and intrinsic noise. Journal of Molecular Evolution. 1984;20(2):135–46. doi:10.1007/BF02257374. PMID 6433032. Bibcode: 1984 JMolE..20.135P. Collier pp. 75–91 Dimmock p. 70

58. Boevink P, Oparka KJ. Virus-host interactions during movement processes. Plant Physiology. 2005;138(4):1815–21. doi:10.1104/pp.105.066761. PMID 16172094. Dimmock p. 71

59. Barman S, Ali A, Hui EK, Adhikary L, Nayak DP. Transport of viral proteins to the apical membranes and interaction of matrix protein with glycoproteins in the

assembly of influenza viruses. Virus Research. 2001;77(1):61–69. doi:10.1016/S0168-1702(01)00266-0. PMID 11451488.

60. Goris N, Vandenbussche F, De Clercq K. Potential of antiviral therapy and prophylaxis for controlling RNA viral infections of livestock. Antiviral Research. 2008; 78(1):170–78. doi:10.1016/j.antiviral.2007.10.003. PMID 18035428.
61. Carmichael L. An annotated historical account of canine parvovirus. Journal of Veterinary Medicine Series B. 2005;52(7–8):303–11. doi:10.1111/j.1439-0450.2005.00868.x. PMID 16316389.
62. Chen YP, Zhao Y, Hammond J, Hsu H, Evans JD, Feldlaufer MF. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. Journal of Invertebrate Pathology. October–November 2004;87(2–3):84–93. doi:10.1016/j.jip.2004.07.005. PMID 15579317.Shors pp. 562–87
63. Wommack KE, Colwell RR. Virioplankton: viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews. 2000;64(1):69–114. doi:10.1128/MMBR.64.1.69-114.2000. PMID 10704475.
64. Bergh O, Børsheim KY, Bratbak G, Heldal M. High abundance of viruses found in aquatic environments. Nature. 1989;340(6233):467–68. doi:10.1038/340467a0. PMID 2755508. Bibcode: 1989Natur.340.467B.
65. Prangishvili D, Garrett RA. Exceptionally diverse morphotypes and genomes of crenarchaeal hyperthermophilic viruses. Biochemical Society Transactions. 2004;32(Pt 2):204–8. doi:10.1042/BST0320204. PMID 15046572.
66. Campbell, N. "Biology:Concepts & Connections". Pearson Education. San Francisco: 2003. "prokaryote". Online Etymology Dictionary.
67. *Coté G, De Tullio M (2010).* "Beyond Prokaryotes and Eukaryotes: Planctomycetes and Cell Organization"*. Nature.*
68. Stanier RY, Van Niel CB *(1962). "The concept of a bacterium". Archiv für Mikrobiologie. 42: 17–35.* doi:10.1007/BF00425185. PMID 13916221.

69. *Bauman RW, Tizard IR, Machunis-Masouka E (2006).* Microbiology. *San Francisco: Pearson Benjamin Cummings.* ISBN 978-0-8053-7693-7.

70. *Stoeckenius W (October 1981).* "Walsby's square bacterium: fine structure of an orthogonal procaryote". *Journal of Bacteriology. 148 (1): 352–60.* PMC 216199. PMID 7287626.

71. *Rosenshine I, Tchelet R, Mevarech M (September 1989). "The mechanism of DNA transfer in the mating system of an archaebacterium". Science. 245 (4924): 1387–9.* Bibcode:1989Sci...245.1387R. doi:10.1126/science.2818746. PMID 2818746.

72. *Adl SM, Simpson AG, Lane CE, Lukeš J, Bass D, Bowser SS, et al. (September 2012).* "The revised classification of eukaryotes" (PDF). *The Journal of Eukaryotic Microbiology. 59 (5): 429–93.* doi:10.1111/j.1550-7408.2012.00644.x. PMC 3483872. PMID 23020233. *Archived from* the original (PDF) *on 16 June 2016.*

73. *Youngson RM (2006). Collins Dictionary of Human Biology. Glasgow: HarperCollins.* ISBN 978-0-00-722134-9.

74. *Nelson DL, Cox MM (2005).* Lehninger Principles of Biochemistry *(4th ed.). New York: W.H. Freeman.* ISBN 978-0-7167-4339-2.

75. *Martin, E.A., ed. (1983). Macmillan Dictionary of Life Sciences (2nd ed.). London: Macmillan Press.* ISBN 978-0-333-34867-3.

76. *Harper, Douglas.* "eukaryotic". Online Etymology Dictionary.

77. *Chatton, Édouard (1937). Titres Et Travaux Scientifiques (1906-1937) De Edouard Chatton. Sète: Impr. E. Sottano.*

78. *Yamaguchi, Masashi; Worman, Cedric O'Driscoll (2014).* "Deep-sea microorganisms and the origin of the eukaryotic cell" (PDF). *Jpn. J. Protozool. 47 (1, 2): 29–48.*

79. *Linka M, Weber AP (2011).* "Evolutionary Integration of Chloroplast Metabolism with the Metabolic Networks of the Cells". *In Burnap RL, Vermaas WF (eds.). Functional*

Genomics and Evolution of Photosynthetic Systems. Springer. p. 215. ISBN 978-9400715332.

80. *Marsh, Mark (2001). Endocytosis. Oxford University Press. p. vii.* ISBN 978-0-19-963851-2.
81. *Hetzer MW (March 2010).* "The nuclear envelope". *Cold Spring Harbor Perspectives in Biology. 2 (3): a000539.* doi:10.1101/cshperspect.a000539. PMC 2829960. PMID 20300205.
82. *Raven J (July 1987). "The role of vacuoles". New Phytologist. 106 (3): 357–422.* doi:10.1111/j.1469-8137.1987.tb00149.x.
83. *Oparka K (2005). Plasmodesmata. Oxford, UK: Blackwell Publishing.*
84. *Deacon J (2005). Fungal Biology. Cambridge, Massachusetts: Blackwell Publishers. pp. 4 and passim.* ISBN 978-1-4051-3066-0.
85. *Keeling PJ (October 2004).* "Diversity and evolutionary history of plastids and their hosts". *American Journal of Botany. 91 (10): 1481–1493.* doi:10.3732/ajb.91.10.1481. PMID 21652304.
86. *David J. Patterson.* "Amoebae: Protists Which Move and Feed Using Pseudopodia". *Tree of Life Web Project*. Retrieved 12 November 2017.
87. *Lane N (June 2011).* "Energetics and genetics across the prokaryote-eukaryote divide". *Biology Direct. 6 (1): 35.* doi:10.1186/1745-6150-6-35. PMC 3152533. PMID 21714941.
88. *Goldfuß (1818).* "Ueber die Classification der Zoophyten"*[On the classification of zoophytes]. Isis, Oder, Encyclopädische Zeitung von Oken (in German). 2 (6): 1008–1019.* From p. 1008: "Erste Klasse. Urthiere. Protozoa." (First class. Primordial animals. Protozoa.)
89. *Scamardella, JM (1999).* "Not plants or animals: a brief history of the origin of Kingdoms Protozoa, Protista and Protoctista" (PDF). International Microbiology. *2: 207–221. Archived from* the original (PDF) *on 14 June 2011.*

90. Rothschild LJ *(1989). "Protozoa, Protista, Protoctista: what's in a name?". Journal of the History of Biology. 22 (2): 277–305.* doi:10.1007/BF00139515. PMID 11542176.

91. *Adl SM, Simpson AG, Farmer MA, Andersen RA, Anderson OR, Barta JR, et al. (2005). "The new higher level classification of eukaryotes with emphasis on the taxonomy of protists". The Journal of Eukaryotic Microbiology. 52 (5): 399–451.* doi:10.1111/j.1550-7408.2005.00053.x. PMID 16248873.

Unit 3: Microbial Metabolism and Growth

Unit Structure

3.0 Learning Objectives

After studying this unit you would be able to:

- Explain metabolism and microbial metabolism
- Describe types of microbial metabolism

- Explain the pattern of microbial cell growth
- Explain particular metabolic properties
- Describe the heterotrophic metabolism in microbes

3.1 Introduction

Each and every biochemical reaction that takes place in a cell or organism is known as metabolism. You can learn about the chemical diversity of substrate oxidations and dissimilation reactions by studying bacterial metabolism. Through these reactions substrate molecules are broken down, to produce energy in bacteria. You also learn about the uptake and use of the inorganic or organic compounds necessary for growth and maintenance. These are known as assimilation reactions. These respective exergonic (energy-yielding) and endergonic (energy-requiring) reactions are catalyzed within the living bacterial cell by integrated enzyme systems, which results in the self-replication of the cell.

The bacterial cell is an extremely specialized energy transformer. Chemical energy produced by substrate oxidations is conserved by formation of high-energy compounds such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

As of metabolic and nutritional standpoint, three main physiologic kind of bacteria are; heterotrophs or chemoorganotrophs, autotrophs or chemolithotrophs and photosynthetic bacteria or phototrophs

3.3 Metabolism

Metabolism is the set of vital chemical reactions in organisms. The three core functions of metabolism are: the conversion of food to energy to carry cellular processes; the conversion of food to building blocks for proteins, lipids, nucleic acids, and some carbohydrates; and the removal of nitrogenous wastes. These reactions which are catalyzed by enzymes permit organisms to grow and reproduce, sustain their structures, and acknowledge to their environments.

Metabolism is also known as the addition of all chemical reactions that take place in living organisms. It includes digestion and the movement of substances into and between various cells.

Metabolism is the combination of two reactions catabolism and anabolism. Catabolism is the breakdown of compounds; example, the breakdown of glucose to pyruvate by cellular respiration. Anabolism is the building up (synthesis) of compounds like proteins, carbohydrates, lipids, and nucleic acids. Generally, energy releases in catabolism, and energy consumes in anabolism.

The chemical reactions of metabolism are controlled by metabolic pathways, in which one chemical is changed all the way through a sequence of steps into a new chemical. Every step speeds up by a particular enzyme. Enzymes perform as catalysts. These permit a reaction to carry on more speedily. Enzymes also regulate the rate of a metabolic reaction.

Animals, plants and microorganisms are made from four fundamental molecules. These are proteins, carbohydrates , nucleic acid and lipids/fats). These molecules are essential for living. In anabolism building up of these molecules takes place during the construction of cells and tissues and in catabolism breaking down and use of these as a source of energy is done, by their digestion. These bio molecules can be united together and build polymers for example DNA and proteins. These are necessary macromolecules of living organisms.

3.4 Types of Metabolic Reactions

3.4.1 Catabolism

Catabolism includes various metabolic processes in which break down of large molecules takes place, for example break down and oxidation of food molecules. The function of the catabolic reactions is to supply energy and components required by anabolic reactions which construct molecules.

Different organisms have different kind of catabolic reactions. Organisms can be classified on the basis of sources of energy and carbon i.e. their primary nutritional groups. The organisms use organic molecules as a source of energy known as organotrophs, whereas lithotrophs use inorganic substrates, and phototrophs use sunlight as chemical energy.

Different types of metabolism depend on redox reactions in which the transfer of electrons from reduced donor molecules such as organic molecules, water, ammonia, hydrogen

sulfide or ferrous ions is done to acceptor molecules such as oxygen, nitrate or sulfate. In animals complex organic molecules are broken down to simpler molecules, for example carbon dioxide and water. In photosynthetic organisms, such as plants and cyanobacteria, electron-transfer reactions do not release energy but are used as a means to store energy absorbed from sunlight.

Digestion

Macromolecules break down into smaller units to be used in cell metabolism. Different enzymes are used to digest these macromolecules. These digestive enzymes consist of proteases that digest proteins into amino acids, glycoside hydrolases digest polysaccharides into monosaccharides. Microorganisms merely secrete digestive enzymes into their surroundings, whereas animals secrete these from the specialized cells of their alimentary canal, stomach, pancreas, and salivary glands.

3.4.2 Anabolism

Anabolism includes constructive metabolic processes in which the energy released by catabolism is used to make complex molecules. In general, the complex molecules that make up cellular structures are constructed step-by-step from small and simple precursors. Anabolism consists of three fundamental phase. First, the production of precursor like amino acids, monosaccharides, isoprenoids and nucleotides. In second step, activation of these precursors into reactive forms takes place. It is done by using energy from ATP. In third phase assemblage of these precursors takes place to form complex molecules like proteins, polysaccharides, lipids and nucleic acids.

In photosynthetic prokaryotes the mechanisms of carbon fixation are more varied. Carbon fixation is done by the Calvin – Benson cycle, a reversed citric acid cycle, or the carboxylation of acetyl-CoA. Prokaryotic chemoautotrophs also fix CO_2 through the Calvin–Benson cycle, but use energy from inorganic compounds to constrain the reaction.

Self-Assessment 1

1. What are the three core functions of metabolism?

2. What is the name of the metabolic processes in which break down of large molecules takes place?

3. What is the name of the constructive metabolic processes in which the energy released by catabolism is used to make complex molecules?

4. Write the name of assimilation reactions takes place within bacterial cells.

5. Animals, plants and microorgnisms are made from which four fundamental molecules?

6. Animals, plants and microorgnisms are made from with four fundamental molecules. These can be united together and build which polymers?

_____

_____

7. Prokaryotic chemoautotrophs fix CO2 through which cycle?

_____

_____

_____

_____

_____

3.5 Microbial Metabolism

Microbial metabolism is the way by which microorganisms get the energy and nutrients. It is required to survive and reproduce. Microorganisms employ various types of metabolic activities. Microbial species can frequently be distinguished from each other on the basis of metabolic characteristics. The particular metabolic properties of a microorganism are the key feature to determine its ecological function, role in industrial processes and biogeochemical cycles.

Metabolism of microorganisms can be as according to the following three principles:

1. Microorganism gets carbon to synthesize cell mass:

- Autotrophic – carbon is acquired from carbon dioxide
- Heterotrophic – carbon is acquired from organic compounds
- Mixotrophic – carbon is acquired from both organic compounds and by fixing carbon dioxide

2. Microorganism gets reducing equivalents (hydrogen atoms or electrons) used either in energy conservation or in biosynthetic reactions:

- Lithotrophic – Reducing equivalents are acquired from inorganic compounds
- Organotrophic – reducing equivalents are acquired from organic compounds

3. Microorganism gets energy for living and growing:

- Phototrophic – energy gets from light
- Chemotrophic – energy gets from external chemical compounds

Some characteristic examples of Metabolism of microorganisms are as follows:

- **Chemolithoautotrophs** get energy from the oxidation of inorganic compounds and carbon from the fixation of carbon dioxide. Examples: Nitrifying bacteria, sulfuoxidizing bacteria, iron-oxidizing bacteria, Knallgas-bacteria
- **Photolithoautotrophs** get energy from light and carbon from the fixation of carbon dioxide, by using reducing equivalents from inorganic compounds. Examples: Cyanobacteria (water as reducing equivalent = hydrogen donor), Chlorobiaceae, Chromatiaceae (hydrogen sulfide as hydrogen donor), Chloroflexus (hydrogen as reducing equivalent donor)
- **Chemolithoheterotrophs** get energy from the oxidation of inorganic compounds, but cannot fix carbon dioxide. Examples: some Thiobacillus, some Beggiatoa, some Nitrobacter spp., Wolinella (with H_2S as reducing equivalent donor), some Knallgas-bacteria, some sulfate-reducing bacteria
- **Chemoorganoheterotrophs** get energy, carbon, and hydrogen for biosynthetic reactions from organic compounds. Examples of bacteria, e.g. Escherichia coli, Bacillus spp., Actinobacteria
- **Photoorganoheterotrophs** get energy from light, carbon and reducing equivalents for biosynthetic reactions from organic compounds. Some species are severely heterotrophic, various others be able to also fix carbon dioxide and are mixotrophic. Examples: Rhodobacter, Rhodopseudomonas, Rhodospirillum, Rhodomicrobium, Rhodocyclus, Heliobacterium, Chloroflexus (alternatively to photolithoautotrophy with hydrogen)

3.6 Energy Transformations in Microorganisms

Energy from Inorganic Compounds

Chemolithotrophy is a type of metabolism in which energy is attained from the oxidation of inorganic compounds. It is present in prokaryotes. Prokaryotes use hydrogen, reduced sulfur compounds (sulfide, hydrogen sulfide and thiosulfate), ferrous iron (FeII) or ammonia as resource of reducing power. They get energy from the oxidation of these compounds with electron acceptors like oxygen or nitrite. These microbial

procedures are significant in acetogenesis, nitrification and denitrification. These are important for fertility of soil.

Energy from light

Sunlight energy is captured by plants, cyanobacteria, purple bacteria, green sulfur bacteria and some protists. They convert carbon dioxide into organic compounds in photosynthesis. Capture of energy and carbon fixation can run separately in prokaryotes. Purple bacteria and green sulfur bacteria use sunlight as a source of energy, while switching between carbon fixation and the fermentation of organic compounds.

3.7 Particular Microbial Metabolic Properties

3.7.1 Methylotrophy

When an organism use C1-compounds as energy sources is known as Methylotrophy. These compounds consist of methanol, methyl amines, formaldehyde, and formate. A number of other less common substrates which lack carbon-carbon bonds may also be used for metabolism. Examples of methylotrophs consist of the bacteria Methylomonas and Methylobacter.

The organisms which use methane (CH_4) as a carbon source are known as Methanotrophs. They oxidize methane consecutively into methanol (CH_3OH), formaldehyde (CH_2O), formate ($HCOO^-$) and carbon dioxide. Enzyme methane monooxygenase is used in this process. As oxygen is required in this process, all methanotrophs are obligate aerobes.

Methane can also be oxidized anaerobically. This occurs by a group of sulfate-reducing bacteria and family of methanogenic Archaea.

Biological production of methane is known as Methanogenesis. It is accomplished by methanogens. These are strictly anaerobic Archaea of which examples are Methanococcus, Methanocaldococcus, Methanobacterium, Methanothermus, Methano sarcina, Methanosaeta and Methanopyrus.

Some methanogens chemolithoautotrophically reduce carbon dioxide to methane using electrons from hydrogen gas. These methanogens can frequently be present in environments having fermentative organisms. The firm alliance of methanogens and fermentative bacteria can be called to be syntrophic.

Some methanogens use methanol as a substrate for methanogenesis. These are chemoorganotrophic. These are autotrophic also because these use CO_2 as only carbon source.

Some methanogens produce both methane and carbon dioxide from acetate.

3.7.2 Syntrophy

Syntrophy is known as coupling of numerous species to attain a chemical reaction that only would be vigorously unfavorable. Oxidation of fermentative end products like acetate, ethanol and butyrate by organisms like Syntrophomonas is the example of this process.

3.7.3 Anaerobic respiration

When respiration occurs in the absence of oxygen, it is known as anaerobic respiration. Although aerobic organisms use oxygen as a terminal electron acceptor, anaerobic organisms use other electron acceptors during respiration. These inorganic compounds have a lower reduction potential than oxygen. This kind of respiration is resourceful in a smaller amount which results in a slower growth rates than aerobes.

Various microorganisms can use either oxygen or alternative terminal electron acceptors for respiration as according to the environmental conditions. These are known as facultative anaerobes.

The majority of respiring anaerobes are heterotrophs. Even though a number of are autotrophs. Assimilative pathways and dissimilative pathways of anaerobic respiration are found.

Dissimilative pathways meaning that these are used during energy production and not to supply nutrients to the cell. In several forms of anaerobic respiration assimilative pathways are also found.

3.7.4 Denitrification

Denitrification is the stepwise reduction of nitrate to nitrite (NO^{-}_2), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric

oxide reductase, and nitrous oxide reductase, respectively. It is an extensive method used by various Proteobacteria.

A few organisms for example E. coli merely produce nitrate reductase. So they can carry out only the first reduction primarily to the buildup of nitrite. Other organisms for example Paracoccus denitrificans or Pseudomonas stutzeri reduce nitrate wholly.

Absolute denitrification is an important procedure in the atmosphere because some intermediates of denitrification (nitric oxide and nitrous oxide) are significant greenhouse gases. These gases react with sunlight and ozone and make nitric acid which is a constituent of acid rain.

Denitrification is moreover significant in biological wastewater treatment. It is used to decrease the quantity of nitrogen released into the atmosphere thus reducing eutrophication. Denitrification can be determined by nitrate reductase test.

3.7.5 Sulfate reduction

Sulfate reduction is found in various Gram-negative bacteria for example within the deltaproteobacteria, Gram-positive bacteria relating to Desulfotomaculum or the archaeon Archaeoglobus. It is a comparatively a less vigorous method. Hydrogen sulfide (H_2S) is the final produce of this metabolic process. For sulfate reduction electron donors and energy are required.

3.7.6 Acetogenesis

Acetogenesis is a kind of microbial metabolism in which hydrogen is used as an electron donor and carbon dioxide as an electron acceptor to generate acetate. Bacteria which are able to produce acetate autotrophically are known as homoacetogens. This pathway is also used for carbon fixation by Autotrophic sulfate-reducing bacteria and hydrogenotrophic methanogens also use this pathway for carbon fixation. homoacetogens may also be fermentative. They use the hydrogen and carbon dioxide produced as a result of fermentation to produce acetate, which is secreted as a final product.

3.7.7 Chemolithotrophy

It is a kind of metabolism where energy is acquired from the oxidation of inorganic compounds. The majority of chemolithotrophic organisms are also autotrophic. There are two main purposes to chemolithotrophy: the production of energy in the form of ATP and of reducing power as NADH.

3.7.8 Hydrogen oxidation

Several organisms can use hydrogen as a source of energy. These are known as hydrogen oxidizing bacteria. These organisms like Cupriavidus necator, habitually live in oxic-anoxic border in the environment to take the benefit of the hydrogen produced by anaerobic fermentative organisms whereas still sustain a supply of oxygen.

3.7.9 Sulfur oxidation

In sulfur oxidation the oxidation of reduced sulfur compounds like Hydrogen sulfide, inorganic sulfur, and thiosulfate exists to form sulfuric acid. A typical example of sulfur-oxidizing bacteria is Beggiatoa. One more example is Paracoccus.

3.7.10 Nitrification

The process through which ammonia is changed in to nitrate is known as Nitrification. Two different processes are involved in it. First is oxidation of ammonia to nitrite by nitrosifying bacteria (e.g. Nitrosomonas) and second is oxidation of nitrite to nitrate by the nitrite-oxidizing bacteria (e.g. Nitrobacter).

Oxygen is necessary in both ammonia and nitrite oxidation. It shows that both nitrosifying and nitrite-oxidizing bacteria are aerobic in nature.

3.7.11 Anammox

Anammox is anaerobic ammonia oxidation. The organisms accountable for it were discovered recently in the late 1990s. This type of metabolism takes place in members of the Planctomycetes. The example of the microorganisms is Candidatus Brocadia anammoxidans. This process involves the union of ammonia oxidation to nitrite reduction. Oxygen is not necessary for this process. These organisms are strictly anaerobic in nature.

3.7.12 Phototrophy

Various microorganisms are able to use light as a source of energy. These are known as phototrophs. They produce ATP and organic compounds such as carbohydrates, lipids, and proteins by using light. Of these, algae are mainly considerable because they are oxygenic. They use water as an electron donor for electron transfer all through photosynthesis. Phototrophic bacteria are found in the phyla Cyanobacteria, Chlorobi, Proteobacteria, Chloroflexi, and Firmicutes. All along with plants these microorganisms are accountable for all biological production of oxygen gas on Earth.

3.7.13 Nitrogen fixation

Every biological organism needs nitrogen for growth and development. It is present 80% by volume in the atmosphere. It is usually biologically difficult to get to because of its high activation energy. Only particular bacteria and Archaea are able for nitrogen fixation. They convert nitrogen into ammonia which is simply assimilated by all organisms. Thus these prokaryotes are ecologically very significant and are frequently necessary for the continued existence of whole ecosystems. This is especially true In the ocean ecosystem nitrogen-fixing cyanobacteria are merely the frequent sources of fixing nitrogen, and in soil ecosystem specific symbiosis be present between legumes and nitrogen-fixing associates to supply the nitrogen required by the plants for growth and development.

Self-assessment 2

1. Through which process microorganisms get the energy and nutrients?

_____
_____
_____
_____
_____

2. Write the examples of sulfur-oxidizing bacteria?

_____
_____
_____
_____
_____

3. Biological production of methane is known as by which name? Give the examples of the bacteria which are involved in this process.

4. The organisms which use methane (CH_4) as a carbon source are known as by which name?

5. Write a short note on Anammox**.**

6. What are phototrophs? Give the examples of phototrophic bacteria.

7. In which microbial metabolism hydrogen is used as an electron donor and carbon dioxide as an electron acceptor to generate acetate?

3.8 Heterotrophic Metabolism in Microbes

Microorganisms use organic compounds as both carbon and energy sources are called heterotrophic. More specifically these are called chemoorganoheterotrophic. Heterotrophic microorganisms live on other organisms and take nutrients from the host. These are known as commensals or parasites. If these are found in dead organic matter, they are known as saprophytes.

Microbial metabolism plays an important role in bodily decompose of all organisms after death. Many eukaryotes are heterotrophic by having predation or parasitism. Some bacteria are an intracellular parasite of other bacteria, results in the death of its victim, Ex. Bdellovibrio. Myxobacteria are predators of other bacteria, Ex. Myxococcus.

The majority of pathogenic bacteria found as heterotrophic parasites of humans or the other eukaryotic organisms. Heterotrophic microbes are found plentiful in nature. They cause breakdown of large organic polymers such as cellulose, chitin or lignin which are usually indigestible to larger animals.

The oxidative breakdown of large polymers to carbon dioxide is known as mineralization. For this process there is the need of numerous types of organisms. Some organisms break down the polymer into its constituent monomers and others use these monomers and excrete simpler waste compounds as by-products. Some organisms use the excreted waste products. Different types of organisms degrade different polymers and secrete different kind of waste products. Some organisms can degrade petroleum compounds or pesticides, so that these can be used in bioremediation.

Biochemically, prokaryotic heterotrophic metabolism is much more versatile than that of eukaryotic organisms, although several prokaryotes have the simple metabolic processes as glycolysis which is also known as EMP pathway. It is for sugar metabolism and the citric acid cycle to degrade acetate. Energy is produced in the form of ATP and reducing power in the form of NADH or quinols. These metabolic pathways are required to conserve various building blocks essential for cell growth.

Various bacteria and archaea have another types of metabolic pathways for example sugar metabolism through the keto-deoxy-phosphogluconate pathway. It is also known as ED pathway and found in Pseudomonas. Several bacteria have pentose phosphate pathway for sugar metabolism. Due to the evolutionary diversity of prokaryotes, these have diverse types of metabolic pathways and can use a huge range of organic compounds in comparison to eukaryotes.

3.9 Various Types of Heterotrophic Metabolism in Microbes:

Heterotrophic metabolism is the biological oxidation of organic compounds, like glucose, to produce ATP and simpler organic (or inorganic) compounds. It is required by the bacterial cell for biosynthetic or assimilatory reactions.

3.9.1 Respiration

Respiration is a sort of heterotrophic metabolism. In this process oxygen is used and 38 moles of ATP are resultant from the oxidation of 1 mole of glucose. It results in the yield of 380,000 calorie energy. Additional 308,000 calorie energy is lost as heat.

Respiration is the process when some organic compound (typically carbohydrate) is oxidized wholly to CO_2 and H_2O. In aerobic respiration, molecular oxygen acts like the terminal acceptor of electrons.

Anaerobic respiration is a heterotrophic form of metabolism. In this process a particular compound other than O_2 acts as a terminal electron acceptor. These acceptor compounds consist of nitrate, sulphate, fumarate, and even CO_2 for methane-producing bacteria.

A number of bacteria shows an exclusive form of respiration known as anaerobic respiration. These heterotrophic bacteria do not nurture anaerobically if a particular chemical constituent is added to the medium. These chemical constituents act as a terminal electron acceptor. In anaerobic respiration, nitrate (NO_3^-), sulphate (SO_4^{2-}), carbon di oxide (CO_2) or fumarate act as terminal electron acceptors. The electron acceptor in anaerobic respiration is as according to the bacteria. If protein or amino acid is oxidized, the end product formed is ammonia.

A huge group of anaerobic respirers are the nitrate reducers. The nitrate reducers are principally heterotrophic bacteria that have a composite electron transport system(s) in which NO_3^- ion acts as a terminal acceptor of electrons.

The methanogens are very well known anaerobic bacteria. These are very susceptible to little concentrations of molecular O_2. Archaebacteria are also typically survive in atypical and deadly surroundings.

3.9.2 Fermentation

Fermentation is a sort of heterotrophic metabolism. In it partial glucose oxidation takes place. A lesser amount of energy is produced in this process. The process supports anaerobic growth.

Fermentation is a type of metabolic process in which an organic substance breaks down into a simpler substance through the action of enzymes. It occurs in the absence of oxygen. In microorganisms, organic nutrients are degraded anaerobically and produce adenosine tri phosphate (ATP).

In fermentation NADH produces NAD^+ and an organic product. The examples of organic products are ethanol, lactic acid, and hydrogen gas (H_2), and often carbon dioxide. Though, additional unusual compounds can be produced by fermentation, like butyric acid and acetone.

In fermentation organic carbon is used as an alternative of oxygen as a terminal electron acceptor. These organisms do not use an electron transport chain to oxidize NADH to NAD^+. Thus these have to have a substitute method to use this reducing power and sustain a supply of NAD^+ for the appropriate execution of regular metabolic pathways (Ex. glycolysis). Fermentative organisms are called as anaerobic because they do not require oxygen. In various organisms fermentation occur under anaerobic conditions and aerobic respiration when oxygen is present. These organisms are known as facultative anaerobes.

3.9.2.1 Growth of Microorganisms

In batch fermentation, the growth medium having nutrients is inoculated with microorganisms. Fermentation continues without adding up fresh growth medium.

In fed-batch fermentation, nutrients are added at short time intervals during fermentation. In batch and fed-batch fermentation, growth medium is not removed until the finish of fermentation process.

In continuous fermentation, as the fermentation continues, fresh growth medium is added continuously. At the same time, an equivalent volume of used up medium having suspended microorganisms is removed. It facilitates the cells to grow optimally and constantly.

3.9.2.1.1 Batch Culture or Batch Fermentation:

A batch fermentation is considered as a closed system. The sterile nutrient culture medium is inoculated with microorganisms in the bioreactor. The optimal physiological conditions (pH, temperature, O_2, agitation etc.) are required incubation. To retain pH, acid or alkali and to minimize foam, anti-foam agents are essentially added. Following six characteristic phases of growth are viewed in batch fermentation under optimal conditions for growth.

Pattern of microbial cell growth in batch culture of batch fermentation

- Lag phase
- Acceleration phase
- Logarithmic (log) phase (exponential phase)
- Deceleration phase
- Stationary phase
- Death phase.

Lag phase: The early short phase of culturing after inoculation is called as lag phase. In this phase, the microorganisms settle in to the new environment as according to the availability of nutrients, pH, moisture, etc. There is no increase in the cell number, while the cellular weight may increase to some extent.

The duration of the lag phase is changeable. It is generally determined by the new set of physiological conditions, and the stage at which the micro-organisms are present when inoculated. For example, if the culture inoculated is at log phase, lag phase may not happen and growth may begin instantly.

Acceleration phase: This is a short temporary stage throughout which cells begin growing gradually. Actuality, acceleration phase join the lag phase and log phase.

Log phase: The most vigorous growth of microorganisms and multiplication happen throughout the log phase. The cells go through a number of doublings and the cell mass increases. When the number of cells or biomass is plotted against time on a semi logarithmic graph, a straight line is attained; therefore the name log phase is called.

In log phase growth rate of microbes is free from substrate (nutrient supply) concentration as long as surplus substrate is present, and there are no growth inhibitors in the medium.

Deceleration phase: As the growth rate of microorganisms during log phase decreases, they enter into the deceleration phase. This phase is generally very transitory and may not be visible.

Stationary phase: As the substrate in the growth medium gets exhausted, and the metabolic end products that are produced reduce the growth, the cells go through the stationary phase. The microbial growth may either reduces or wholly bring to an end. The biomass may stay approximately constant during stationary phase. In this phase significant changes in the metabolism of the cells may occur which may produce secondary metabolites of biotechnological importance e.g. production of antibiotics.

Death phase: This phase is related with termination of metabolic activity and reduction of energy reserves. The cells die at an exponential rate i.e. a straight line may get when the number of surviving cells is plotted against time on a semi logarithmic plot. In the commercial and industrial fermentations, the growth of the microorganisms is stopped at the end of the log phase or immediately prior to the death phase starts, and the cells are produced.

3.9.2.1.2 Fed-Batch Culture or Fed-Batch Fermentation:

Fed-batch fermentation is an upgrading of batch fermentation in which the substrate is added in addition at various times all through the path of fermentation. In batch culture method, substrate is added only at the start of the fermentation. Periodical addition of substrate extends log and stationary phases, results in an increased biomass. As a result, production of metabolites (e.g. antibiotics) during stationary phase is very much improved.

In fed-batch fermentation direct calculation of substrate concentration is complicated so other indicators that link with substrate consumption are used. The formation of organic acids, production of CO_2 and changes in pH may be calculated, and consequently substrate addition is done. Commonly, fed-batch fermentation needs additional cautious supervise than batch fermentation. Thus this method is not preferred by industries.

Fed-batch fermentation for the production of recombinant proteins:

In current years, fed-batch fermentation has turned into popular due to very elevated yield, for the production of recombinant proteins. The product yield may increases from 25% to 100% in fed-batch fermentation in contrast to batch fermentation. It depends on the microorganism and the nature of recombinant protein. Alert observation of the fermentation

reaction and suitable adding up of substrates (carbon and nitrogen sources, and trace metals) significantly increases yield of the product.

Limitations: The main limitation of fed batch fermentation is that the microorganisms in the stationary phase generate proteolytic enzymes or proteases. These enzymes attack the recombinant proteins that are being produced. By cautious observation of the fermentation, the log phase can be extended and the beginning of stationary phase is postponed. By this way, the formation of proteases can be reduced.

3.9.3 Bacterial Photosynthesis

Bacterial photosynthesis depends on light. It is a sort of anaerobic metabolism. Glucose is formed due to the reduction of Carbon dioxide, which is used for both biosynthesis and energy production. As according to the hydrogen source used to reduce CO_2, both photolithotrophic and photoorganotrophic reactions be present in bacteria.

Various prokaryotes (bacteria and cyanobacteria) have phototrophic forms of metabolism. The modes of photosynthesis in these prokaryotes vary primarily in the sort of compound that acts as the hydrogen donor in the reduction of CO_2 to glucose. Phototrophic organisms make use of the glucose synthesized intracellularly for biosynthetic use (for example: starch synthesis) or for production of energy, which typically happens all the way through cellular respiration.

3.9.4 Autotrophy

Autotrophy is a distinctive kind of metabolism which is present only in bacteria. Inorganic compounds are oxidized directly (without using sunlight) to yield energy (e.g., NH_3, NO_2^-, S_2, and Fe^{2+}). This kind of metabolism also necessitates energy to reduce CO_2, similar to photosynthesis. Although no lipid mediated processes are implicated. This type of metabolism is also known as chemotrophy, chemoautotrophy, or chemolithotrophy.

Bacteria that nurture exclusively by utilizing inorganic compounds (mineral ions), exclusive of using sunlight as an energy supply, are known as autotrophs, chemotrophs, chemoautotrophs, or chemolithotrophs. The entire autotrophs use CO_2 as a carbon source for growth. They get nitrogen from inorganic compounds like ammonia, nitrate and nitrogen.

Sulfur-oxidizing or sulfur-compound-oxidizing bacteria are also found as autotrophic microorganisms. These bacteria oxidize H_2S, S_2, and S_2O_3. Among the sulfur bacteria are two autotrophs; Thiobacillus ferrooxidans, obtains energy by oxidizing elemental sulfur or ferrous iron, and T. denitrificans, obtains energy by oxidizing S_2O_3 anaerobically. They utilize NO_3^- as the exclusive terminal electron acceptor. T denitrificans reduces NO_3 to molecular N_2, which is liberated as a gas. This process is known as denitrification.

3.9.5 Anaerobic Respiration

Anaerobic respiration is a heterotrophic form of metabolism. In this process a particular compound other than O_2 acts as a terminal electron acceptor. These acceptor compounds consist of nitrate, sulphate, fumarate, and even CO_2 for methane-producing bacteria.

A number of bacteria shows an exclusive form of respiration known as anaerobic respiration. These heterotrophic bacteria do not nurture anaerobically if a particular chemical constituent is added to the medium. These chemical constituents act as a terminal electron acceptor.

A huge group of anaerobic respirers are the nitrate reducers. The nitrate reducers are principally heterotrophic bacteria that have a composite electron transport system(s) in which NO_3^- ion acts as a terminal acceptor of electrons.

The methanogens are very well known anaerobic bacteria. These are very susceptible to little concentrations of molecular O_2. Archaebacteria are also typically survive in atypical and deadly surroundings.

3.9.6 Nitrogen Cycle

This is a metabolic method which fixes atmospheric nitrogen to the soil. Bacteria, plants, and animals are involved in this process. Organic and inorganic nitrogen compounds are used metabolically and recycled. All the fundamental chemical elements (S, O, P, C, and H) necessary to sustain living organisms have geochemical cycles alike to the nitrogen cycle.

The nitrogen cycle is an ultimate example of the ecologic interdependence of bacteria, plants, and animals. Nitrogen is recycled when organisms utilize one form of nitrogen for growth and excrete another nitrogenous compound as a waste product. This waste product is utilized by another type of organism as a growth or energy substrate.

Important processes consist of ammonification, mineralization, nitrification, denitrification, and nitrogen fixation, are carried out primarily by bacteria.

Ammonification is the process in which the particular breakdown of organic nitrogenous compounds takes place. In it proteins are degraded to amino acids (proteolysis) and then to inorganic NH_3 by heterotrophic bacteria. This is a necessary step in the nitrogen cycle. The breakdown of feces and urine also happens by ammonification.

Mineralization is the process in which at demise the organic constituents of the tissues and cells decompose biologically to inorganic constituents. These constituents act as nutrients for other life forms. The NH_3 liberated in turn acts as an utilizable nitrogen source for various other bacteria.

In nitrification the change of NH_3 to NO_3 by autotrophes in the soil; in denitrification the anaerobic conversion of NO_3 to N_2 gas takes place by various heterotrophs. Nitrogen fixation is the conversion of $N_2$to NH_3 and cell protein. The concluding is an extremely particular prokaryotic procedure known as diazotrophy, carried out by both free-living bacteria for example Azotobacter, Derxia, Beijeringeia, and Azomona species and symbionts for example Rhizobium species in combination with legume plants for example soybeans, peas, clover, and bluebonnets. All plants depend greatly on NO_3^- as a nitrogen source, and the majority of animals depend on plants for getting nutrients.

Self-assessment 3

1. Explain in short the heterotrophic microorganisms.

_____
_____
_____
_____
_____

2. In various bacteria and archaea sugar metabolism takes place through which pathway?

_____
_____
_____
_____
_____

3. In which process an organic substance breaks down into a simpler substance through the action of enzymes and in the absence of oxygen?

_____
_____
_____
_____
_____

4. Explain in short the fed-batch fermentation and continuous fermentation?

_____
_____
_____
_____
_____

5. Write a short note on autotrophy.

_____
_____
_____
_____
_____

6. What are nitrate reducer bacteria?

_____
_____
_____
_____
_____

7. Which fundamental chemical elements are necessary to sustain living organisms?

_____
_____
_____
_____
_____

Summary

- Metabolism is the set of vital chemical reactions in organisms.
- The three core functions of metabolism are: the conversion of food to energy to carry cellular processes; the conversion of food to building blocks for proteins, lipids, nucleic acids, and some carbohydrates; and the removal of nitrogenous wastes.
- Metabolism is the combination of two reactions catabolism and anabolism.

- Catabolism is the breakdown of compounds; example, the breakdown of glucose to pyruvate by cellular respiration.
- Anabolism is the building up (synthesis) of compounds like proteins, carbohydrates, lipids, and nucleic acids. Generally, energy releases in catabolism, and energy consumes in anabolism.
- Microbial metabolism is the way by which microorganisms get the energy and nutrients.
- Metabolism of microorganisms can be as according to the following three principles:
- Microorganism gets carbon to synthesize cell mass
- Microorganism gets reducing equivalents (hydrogen atoms or electrons) used either in energy conservation or in biosynthetic reactions
- Microorganism gets energy for living and growing
- Energy transformation in microorganisms is through inorganic compounds and light
- When an organism use C1-compounds as energy sources is known as Methylotrophy. These compounds consist of methanol, methyl amines, formaldehyde, and formate.
- Syntrophy is known as coupling of numerous species to attain a chemical reaction that only would be vigorously unfavorable.
- When respiration occurs in the absence of oxygen, it is known as anaerobic respiration.
- Denitrification is the stepwise reduction of nitrate to nitrite (NO^{-}_{2}), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, respectively.
- Acetogenesis is a kind of microbial metabolism in which hydrogen is used as an electron donor and carbon dioxide as an electron acceptor to generate acetate.
- In sulfur oxidation the oxidation of reduced sulfur compounds like Hydrogen sulfide, inorganic sulfur, and thiosulfate exists to form sulfuric acid.

- Various microorganisms are able to use light as a source of energy. These are known as phototrophs.
- Fermentation is a sort of heterotrophic metabolism. In it partial glucose oxidation takes place. A lesser amount of energy is produced in this process. The process supports anaerobic growth.
- The nitrogen cycle is an ultimate example of the ecologic interdependence of bacteria, plants, and animals.

- This is a metabolic method which fixes atmospheric nitrogen to the soil. Bacteria, plants, and animals are involved in this process.
- Important processes consist of ammonification, mineralization, nitrification, denitrification, and nitrogen fixation, are carried out primarily by bacteria.

Terminal Questions

1. Explain in detail what is metabolism and types of metabolic reactions?

2. What is microbial metabolism? Explain in detail the three principles of it.

3. Explain in detail the methylotrophy and syntrophy.

4. Explain in detail the chemolithotrophy and phototrophy.

5. Describe the fermentation and pattern of microbial cell growth in batch culture of batch fermentation.

6. Explain nitrogen cycle and various processes involved in it.

7. What is respiration? Explain aerobic and anaerobic respiration.

8. Define various microbial metabolic properties.

9. How energy transformation occurs in microorganisms?

Answers

Self-assessment 1

1. Refer to section 3.3
2. Refer to section 3.4.1
3. Refer to section 3.4.2
4. Refer to section 3.1
5. Refer to section 3.3
6. Refer to section 3.3
7. Refer to section 3.4.2

Self-assessment 2

1. Refer to section 3.5
2. Refer to section 3.7.9
3. Refer to section 3.7.1
4. Refer to section 3.7.1
5. Refer to section 3.7.11
6. Refer to section 3.7.12
7. Refer to section 3.7.6

Self-assessment 3

1. Refer to section 3.8
2. Refer to section 3.8
3. Refer to section 3.9.2
4. Refer to section 3.9.2.1
5. Refer to section 3.9.4
6. Refer to section 3.9.5
7. Refer to section 3.9.6

Terminal Questions

1. Refer to section 3.3, 3.4
2. Refer to section 3.5
3. Refer to section 3.7.1, 3.7.2
4. Refer to section 3.7.7, 3.7.12
5. Refer to section 3.9.2, 3.9.2.1
6. Refer to section 3.9.6
7. Refer to section 3.9.1, 3.9.5
8. Refer to section 3.7
9. Refer to section 3.6

References

Friedrich C (1998). Physiology and genetics of sulfur-oxidizing bacteria. Adv Microb Physiol. Advances in Microbial Physiology. 39. pp. 235–89. doi:10.1016/S0065-2911(08)60018-1. ISBN 978-0-12-027739-1. PMID 9328649.

Cooper, Geoffrey M. (2000). "The Molecular Composition of Cells". The Cell: A Molecular Approach. 2nd edition.

Jump up to:[a] [b] Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Raff, Martin; Roberts, Keith; Walter, Peter (2002). "How Cells Obtain Energy from Food". Molecular Biology of the Cell. 4th edition – via NCBI.

Raven, Ja (3 September 2009). "Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments". Aquatic Microbial Ecology. 56: 177–192. doi:10.3354/ame01315. ISSN 0948-3055.

Nelson N, Ben-Shem A (2004). "The complex architecture of oxygenic photosynthesis". Nat Rev Mol Cell Biol. 5 (12): 971–82. doi:10.1038/nrm1525. PMID 15573135.

Madigan, Michael T., Martinko, John M (2006). Brock Mikrobiologie (11., überarb. Aufl ed.). München: Pearson Studium. pp. 604, 621. ISBN 3-8273-7187-2. OCLC 162303067.

Demirel, Yaşar, (2016). Energy : production, conversion, storage, conservation, and coupling (Second ed.). Lincoln: Springer. p. 431. ISBN 978-3-319-29650-0. OCLC 945435943.

Häse C, Finkelstein R (December 1993). "Bacterial extracellular zinc-containing metalloproteases". Microbiol Rev. 57 (4): 823–37. doi:10.1128/MMBR.57.4.823-837.1993. PMC 372940. PMID 8302217 – via ASM.

Gupta R, Gupta N, Rathi P (2004). "Bacterial lipases: an overview of production, purification and biochemical properties". Appl Microbiol Biotechnol. 64 (6): 763–81. doi:10.1007/s00253-004-1568-8. PMID 14966663 – via Springer.

Hoyle T (1997). "The digestive system: linking theory and practice". Br J Nurs. 6 (22): 1285–91. doi:10.12968/bjon.1997.6.22.1285. PMID 9470654 – via Mag Online Library.

Morris, J. et al. (2019). "Biology: How Life Works", 3rd edition, W. H. Freeman. ISBN 978-1319017637

Tang, K.-H., Tang, Y. J., Blankenship, R. E. (2011). "Carbon metabolic pathways in phototrophic bacteria and their broader evolutionary implications" Frontiers in Microbiology **2**: Atc. 165. http://dx.doi.org/10.3389/micb.2011.00165

Schmidt-Rohr, K. (2020). "Oxygen Is the High-Energy Molecule Powering Complex Multicellular Life: Fundamental Corrections to Traditional Bioenergetics ACS Omega **5**: 2221-2233.http://dx.doi.org/10.1021/acsomega.9b03352

https://courses.lumenlearning.com/boundless-microbiology/chapter/chemolithotrophy/

Jugder, Bat-Erdene; Welch, Jeffrey; Aguey-Zinsou, Kondo-Francois; Marquis, Christopher P. (2013). "Fundamentals and electrochemical applications of [Ni–Fe]-uptake hydrogenases". RSC Advances. **3** (22): 8142. doi:10.1039/c3ra22668a. ISSN 2046-2069.

Strous M, Fuerst JA, Kramer EH, et al. (July 1999). "Missing lithotroph identified as new planctomycete"(PDF). Nature. **400** (6743): 446–9. Bibcode:1999Natur.400..446S. doi:10.1038/22749. PMID 10440372.

Gräber, Peter; Milazzo, Giulio (1997). Bioenergetics. Birkhäuser. p. 80. ISBN 978-3-7643-5295-0.

Bryant DA, Frigaard NU (November 2006). "Prokaryotic photosynthesis and phototrophy illuminated". Trends Microbiol. **14** (11): 488–96. doi:10.1016/j.tim.2006.09.001. PMID 16997562.

Cabello P, Roldán MD, Moreno-Vivián C (November 2004). "Nitrate reduction and the nitrogen cycle in archaea". Microbiology. **150** (Pt 11): 3527–46. doi:10.1099/mic.0.27303-0. PMID 15528644.

Buchanan RE, Cibbons NE (eds): Bergey's Manual of Determinative Bacteriology. 8th Ed. Williams & Wilkins, Baltimore, 1974 .

Green DE. A critique of the chemosmotic model of energy coupling. Proc Natl Acad Sci USA. 1981;78:2249.

Haddock BA, Hamilton WA (eds): Microbial energetics. 27th Symposium of the Society of General Microbiology. Cambridge University Press, Cambridge, 1977.

Hempfling WP: Microbial Respiration. Benchman Papers in Microbiology no. 13.

Downden, Hutchinson and Ross, Stroudsburg, PA, 1979.

Hill R: The biochemists' green mansions: the photosynthetic electron-transport chain in plants. In Campbell PN, Greville CD (eds): Essays in Biochemistry. Vol.1. Academic Press, New York, 1965.

Jurtshuk P Jr, Liu JK. Cytochrome oxidase and analyses of *Bacillus* strains: existence of oxidase-positive species. Int J Syst Bacterol. 1983;33:887.

Jurtshuk P Jr, Mueller TJ, Acord WC. Bacterial terminal oxidases. Crit Rev Microbiol. 1975;3:359.

Jurtshuk P Jr, Mueller TJ, Wong TY. Isolation and purification of the cytochrome oxidase of *Azotobacter vinelandii*. Biochim Biophys Acta. 1981;637:374.

Jurtshuk P, Jr, Yang TY: Oxygen reactive hemoprotein components in bacterial respiratory systems. In Knowles CJ (ed): Diversity of Bacterial Respiratory Systems. Vol. 1. CRC Press, Boca Raton, FL, 1980 .

Kamp AF, La Riviere JWM, Verhoeven W (eds): Jan Albert Kluyver: His Life and Work. Interscience, New York, 1959 .

Kluyver JA, Van Niel CB: The microbe's contribution to biology. Harvard University Press, Cambridge, MA, 1956 .

Kornberg HL: The role and maintenance of the tricarboxylic acid cycle in *Escherichia coli*. In Goodwin TW (ed): British Biochemistry Past and Present. Biochemistry Society Symposium no. 30. Academic Press, London, 1970 .

Lemberg R, Barrett J: Bacterial cytochromes and cytochrome oxidases. In Lemberg R, Barrett J: Cytochromes. Academic Press, New York, 1973 .

Mandelstam J, McQuillen K, Dawes I (eds): Biochemistry of Bacterial Growth. 3rd Ed. Blackwell, Oxford, 1982 .

O'Leary WM: The chemistry and metabolism of microbial lipids. World Publishing Co, Cleveland, 1967 .

Schlegel HG, Bowier B (eds): Autotrophic Bacteria. Science Tech, Madison, WI, 1989 .

Slepecky RA, Leadbetter ER: Ecology and relationships of endospore-forming bacteria: Changing perspectives. In Piggot P, Moran Jr, CP and Youngman P (eds). Regulation of Bacterial Differentiation. Am Soc Microbiol Press, 1994 .

Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev. 1977;41:100.

Thimann KV: The Life of Bacteria. 2nd Ed. Macmillan, New York, 1966.

Unit 4: Aquatic microbiology

Unit Structure

4.0 Learning Objectives

After studying this unit you would be able to:

- Explain aquatic Ecosystem and its microbiology
- Describe ecotoxicology and why taken up as a unit of study
- Explain toxicants and toxicity
- Describe the factors influencing toxicity
- Explain the testing organisms and toxicity testing

4.1 Introduction

In unit 3 you studied about the microbial metabolism and growth. In this unit you will study about the aquatic microbiology.

Aquatic microbiology is the study of microbes of aquatic systems. It includes microscopic plants, animals, bacteria, viruses and fungi and their behaviour, relation with other organisms in aquatic environment.

Bacteria, virus and fungi are found throughout the aquatic environment. These can be found in fresh and marine ecosystems.

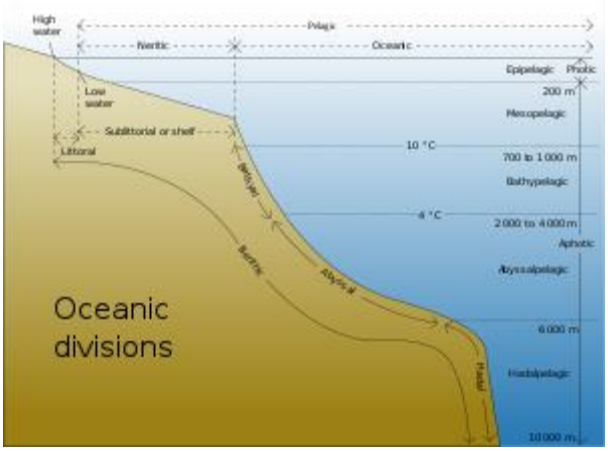
You will also study about ecotoxicology, environmental toxicology and aquatic toxicology. There are two terms, ecotoxicology and environmental toxicology. They vary from each other as toxicity affects to the all levels of biological group from the molecular to the entire population and ecosystems in ecotoxicology, while environmental toxicology consists of toxicity to humans and frequently centralizes its effects at the organism/individual level and below. Further in the unit you will find aquatic toxicology.

4.3 Introduction of Aquatic Environment: Aquatic ecosystem

The ecosystem of water is known as an aquatic ecosystem. Various types of organisms are found in aquatic ecosystems. Two key aquatic ecosystems are marine ecosystems and freshwater ecosystems.

4.3.1 Types of Aquatic Ecosystem

Marine: Marine ecosystem is the biggest of the entire ecosystems; it covers up roughly 71% of the surface of the earth. It includes about 97% of the water of the planet. It produces 32% of the total primary production of the world. It is differentiated from freshwater ecosystems because of the presence of dissolved compounds, particularly salts, in the water. Roughly 85% of the dissolved materials in seawater are sodium and chlorine. Seawater has an average salinity of 35 parts per thousand of water. Actual salinity varies amongst different types of marine ecosystems.



A classification of marine habitats

Marine ecosystems can be divided into various zones depending upon the deepness of water and shoreline features. The oceanic zone is the immense open component of the ocean where animals such as whales, sharks, and tuna exist. The benthic zone is below water where numerous invertebrates reside. The intertidal zone is the region between high and low tides. Added near-shore (neritic) zones contain estuaries, salt marshes, coral reefs, lagoons and mangrove swamps. In the deep water, hydrothermal vents may occur where chemosynthetic sulfur bacteria form the foundation of the food web.

Various classes of organisms exist in marine ecosystems consist of brown algae, dinoflagellates, corals, cephalopods, echinoderms, and sharks. The largest source of commercial foods found in marine ecosystem is fishes.

Freshwater:

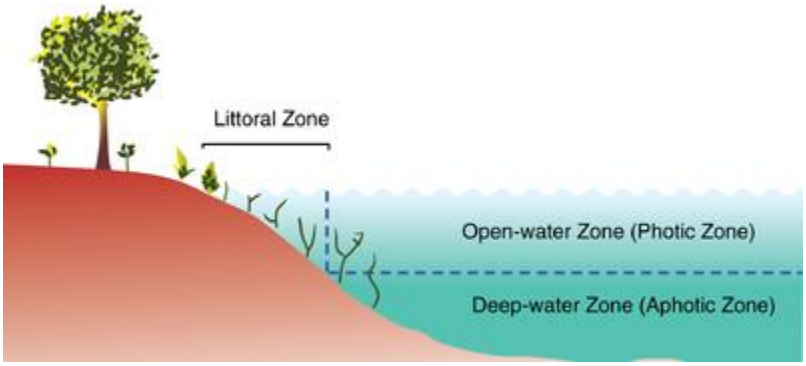
Freshwater ecosystem

Freshwater ecosystems cover up 0.78% of the Earth's surface. It occupies 0.009% of its whole water. It produces almost 3% of its total primary production. Freshwater ecosystems include 41% of the world's well-known species of fish.

There are three fundamental types of freshwater ecosystems:

- Lentic: This type of ecosystem is of slow moving water, including pools, ponds, and lakes.
- Lotic: It is of faster moving water, includes streams and rivers.
- Wetlands: It is of areas where the soil is saturated or flooded for at least part of the time.

Lentic



The three primary zones of a lake.

Lake ecosystems can be divided into zones. One common system divides lakes into three zones. The first zone is the littoral zone. It is the shallow zone close to the shoreline. In this area rooted wetland plants are found. The offshore is divided into two more zones, an open water zone and a deep water zone. In the open water zone (or photic zone) sunlight is present. So that photosynthetic algae is found. In the deep water zone sunlight does not exist and the food web is based on debris incoming from the littoral and photic zones.

Wetlands can be part of the lentic system. Frequently dead trees accrue in this zone. Either due to windfalls on the beach or logs transported to the place for the duration of floods. These wooded remains make available essential habitation for fish and nesting birds, as well as shield shorelines from erosion.

Two significant subclasses of lakes are ponds and water reservoirs.

Ponds: Ponds are small bodies of freshwater. These have shallow and immobile water. These have marsh, and aquatic plants. They can be divided into four zones: vegetation zone, open water, bottom mud and surface film. The size and deepness of ponds frequently

changes significantly with the time. Food web of pond has free-floating algae , aquatic plants, snails, fish, beetles, water bugs, frogs, turtles, otters and muskrats.

Lotic: The chief zones in river ecosystems are decided by bed's gradient or by the velocity of the current of the river. More rapidly moving turbulent water characteristically contains larger concentrations of dissolved oxygen. Due to which larger biodiversity is found than the slow moving water of pools. These differences are the base for the division of rivers into upland and low land rivers.

Wetlands: Wetlands are conquered by vascular plants. They have modified as according to saturated soil. The four major kinds of wetlands are: swamp, marsh, fen and bog (both fens and bogs are types of mire). Wetlands are the most prolific natural ecosystems in the world because they are close to water and soil. Therefore huge numbers of plant and animal species are found there. Due to their good yield, wetlands are frequently transformed into dry land with dykes and drains and used for agricultural purpose.

Self-assessment 1

1. What is aquatic ecosystem?

_____
_____
_____
_____
_____

2. Write the names of various zones of marine ecosystem.

_____
_____
_____
_____
_____

3. Write the names of two key aquatic ecosystems.

_____
_____
_____
_____
_____

4. Write the names of fundamental types of freshwater ecosystems

5. Write the names of the primary zones of a lake.

6. Write the names of the four major kinds of wetlands.

7. What are the two significant subclasses of lakes?

4.3.2 Components of an Aquatic Ecosystem:

4.3.2.1 Abiotic characteristics

An ecosystem has two components: Biotic and Abiotic factors. Biotic factors contains biological interactions among various species of plants and animals. Abiotic contains environmental factors such as substrate type, water deepness, nutrient levels, temperature, moisture, salinity and flow.

The quantity of dissolved oxygen in a water body is commonly the major substance in deciding the amount and types of organic life in the water body. Dissolved oxygen is required by fish to stay alive, though their tolerance to low oxygen varies among species. In severe cases of low oxygen some fish even have alternative to gulp the air. Plants frequently have aerenchyma. On the contrary, oxygen is lethal to numerous kinds of anaerobic bacteria.

Nutrient levels are significant in controlling the profusion of various species of algae. The comparative profusion of nitrogen and phosphorus decide the species of algae found in largest quantity. Algae are incredibly significant supply of food for aquatic life, but if they grow to be plentiful, they may be the reason of decline in the number of fish when they perish. Similarly much more loads of algae in coastal regions, make a hypoxic region of water known as a dead zone, upon decay.

The types of species present in the water body also depend on its salinity. Organisms found in marine ecosystems bear salinity, whereas many freshwater organisms are intolerant of salt.

4.3.2.2 Biotic characteristics

The organisms that are found in an ecosystem determine its biotic characteristics. For example, dense wetland plants cover up huge areas of sediment, or snails or geese may graze the vegetation leaving big mud flats.

Aquatic ecosystems have comparatively low level of oxygen. So that organisms adapt themselves as according to their environment. For example, many aquatic plants have to generate aerenchyma to transmit oxygen to roots.

Autotrophic organisms: Organisms which produce their own food themselves through photosynthesis are called autotrophs. Trees come under this category. They are called as producers that make organic compounds from inorganic material. Algae use solar energy to produce biomass from carbon dioxide and are probably the most important autotrophic organisms in aquatic ecosystem. Rooted and floating vascular plants produce biomass in shallow water.

Chemosynthetic bacteria nourish on hydrogen sulfide in water that comes from volcanic vents. They are found in benthic marine ecosystems. Huge number of animals that feed on these bacteria is found around volcanic vents.

Heterotrophic organisms: Heterotrophic organisms consume autotrophic organisms and use the organic compounds in their bodies as energy sources and as raw materials to create their own biomass. The organisms which are salt tolerant and can stay alive in marine ecosystems, known as Euryhaline organisms, Whereas the organisms which are salt intolerant known as stenohaline, can only exist in freshwater ecosystem.

4.3.2.3 Functions of Aquatic Ecosystem

Aquatic ecosystems carry out various significant environmental functions. For example, they recycle nutrients, purify water, lessen floods, revive ground water and offer habitats for wildlife. Aquatic ecosystems are also used for leisure, and are very important to the tourism industry, particularly in coastal areas.

The vigor of an aquatic ecosystem is ruined when the ecosystem's capacity to soak up a hassle/stress has been surpassed. A stress on an aquatic ecosystem may be due to physical, chemical or biological changes of the environment. Physical changes consist of changes in temperature, water flow and light availability of water. Chemical changes consist of alteration in the loading rates of biostimulatory nutrients, oxygen consuming resources and toxins.

4.4 Aquatic Microbiology

Aquatic microbiology is the study of microbes found in aquatic system like fresh or marine water systems. It consists of microscopic plants, animals, bacteria, viruses and fungi. It includes the study of their behaviour and relation with other organisms in aquatic environment.

Various microorganisms are present in fresh and marine water bodies. The examples of these are bacteria, cyanobacteria, protozoa, algae, and minute animals for example rotifers. These play a significant role in the food chain. For example, cyanobacteria convert solar energy into the energy they require to survive. These cyanobacteria found abundantly in aquatic ecosystem and are used as food for other organisms. The algae that bloom in water are also a significant food supply for other organisms.

Various types of microorganisms exist in fresh water. The area of a water body close to the seashore is known as the littoral zone. It is well lighted, shallow, and warmer than other areas of the water body. Photosynthetic algae and bacteria which use light as energy flourish in this zone.

Next is the limnitic zone, far from the seashore. Sunlight in this zone is only in the upper 100 feet which results in colder. Photosynthetic microbes also exist here. As the water gets deeper, temperatures lowers down results in colder. The concentration of oxygen and light

in the water reduces. Microorganisms which need oxygen do not flourish in this zone. Purple and green sulfur bacteria, which can survive in the absence of oxygen, are found.

Lastly is the benthic zone, found at the bed of fresh water, a small number of microbes live. Bacteria which can exist in the absence of oxygen and sunlight are found, for example Methane producing bacteria.

Microorganisms live at every depth in the sea. These may found even at the ocean floor, thousands of feet underneath the surface.

Microorganisms get dissimilar atmosphere to grow in marine ecosystem in comparison to fresh water. Higher salt concentration, pH and lesser nutrients, comparative to freshwater are poisonous to various microorganisms. Salt loving bacteria flourish close to surface. These are known as halophilic bacteria. Some freshwater bacteria are also found abundantly, examples are Pseudomonas and Vibrio. Archaebacteria also found abundantly in the marine ecosystem. The function of archaebacteria in the food chain of marine ecosystem is not known up till now, but must be of fundamental significance.

A further microorganism present in marine ecosystem is dinoflagellelates. This is a kind of algae. The quick growth and development of dinoflagellates be able to turn the water red. This is called as “red tide”. It may reduce the water of nutrients and oxygen of the water, results in the death of a lot of fishes. Humans being also may become sick by consumption of infected fish.

4.5 Ecotoxicology

Ecotoxicology is the study of the effect of toxic chemicals on biological organisms particularly at the population, society, ecosystem, and biosphere levels. Ecotoxicology is a multifaceted area, which amalgamate toxicology and ecology.

The eventual objective of ecotoxicology is to tell and calculate the effects of pollution within the perspective of all other environmental factors. Based on this knowledge the most efficient and effective action to prevent or remediate any detrimental effect can be identified. If any ecosystem is already affected by pollution, we can have the way to re-establish ecosystem services, structures, and functions competently and successfully by ecotoxicology.

4.5.1 History

The term "ecotoxicology" was contrived by René Truhaut in 1969. He explained it as "the branch of toxicology which contains the toxic effects, caused by natural or synthetic pollutants, to the basics of ecosystems, animals, human, vegetable and microbes, in an integral fundamental perspective"

Even though it was primarily concentrated on the study of synthetic chemicals such as polychlorinated biphenyls (PCBs) and naturally occurring chemicals that are usually released by human activities (chlorine, ammonia, copper, etc.), now is used to explain studies of the ecological effects of various abiotic and biotic agents.

An example of an existing ecotoxicological trouble is ocean acidification. It is due to the amplified dissolution of carbon dioxide into the surface waters of the oceans and interfering with ionic parameter in marine organisms.

4.5.2 Regular Environmental Toxicants

Environmental toxicants	**Found In**
PCBs (polychlorinated biphenyls)	Coolant and insulating fluids, pesticide extenders, adhesives, and hydraulic fluids
Pesticides	commercially grown fruits, vegetables, and meats Example: Methyl parathion
Mold and other mycotoxins.	
Phthalates	Plastic wrap, plastic bottles, and plastic food storage containers
VOCs (volatile organic compounds)	Drinking water and sewage systems Example: Formaldehyde
Dioxins	formed due to burning of wood, coal, and oil
Asbestos	Insulation of flows, ceilings, water pipes, and heating ducts.
Heavy metals	fish, and pesticides Example: Arsenic, mercury, lead, aluminum, and cadmium
Chloroform	

4.5.3 Aquatic Toxicants

Chemicals consist of in the group of aquatic toxicity characterize a significant harm to living organisms and human health all the way through aquatic contact. The harmful effects of these chemicals may be damage to the reproductive, immune, endocrine and/or nervous systems, cancer and even death.

European Union has water policies which act as according to the principles declared in Directive 2000/60/EC. These policies have given the list of priority harmful chemicals. Removal or replacement has to be suggested according to the principles stated in Directive 2000/60/EC about "*the cessation or phasing-out of discharges, emissions and losses of the substances, including an appropriate timetable for doing so. The timetable shall not exceed 20 years after the adoption of these proposals by the European Parliament and the Council*".

The discharge of each of these substances into waterways has to be avoided.

Classification

In European Union To classify aquatic toxic substances, previous Dangerous Substances Directive, known as DSD (67/548/EEC) and the latest Regulation 1272/2008 (commonly known as CLP) lay down pictograms and the following risk phrases ® and hazard statements (H):

i) R50 Very toxic to aquatic organisms

ii) R51 Toxic to aquatic organisms

iii) R53 may cause long-term adverse effects in the aquatic environment

iv) H400 Very toxic to aquatic life

v) H410 Very toxic to aquatic life with long lasting effects

vi) H411 Toxic to aquatic life with long lasting effects

Further governmental institutions have placed priority lists of this type of substances:

EU Water Directive

The list consists of priority hazardous chemicals as according to the Decision No 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 set up the list of priority substances in the area of water policy and revised Directive 2000/60/EC.

Substances dangerous to water (German Federal Ministry of the Environment)

The list consists of substances hazardous to water according to the German Federal Ministry of the Environment that categorized chemicals by means of their hazard level as follows:

Nwg	not dangerous to water
1.	low danger to water
2.	dangerous to water
3.	highly dangerous to water

Regulation (EC) No 166/2006 relating to the founding of a European Pollutant Release and Transfer Register (PRTR) annex II set up a listing of pollutants to water.

4.5.4 Related Legislation and Policies

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Decision No 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC.

i) Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration.

ii) Directive 2008/32/EC of the European Parliament and of the Council of 11 March 2008 amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, as regards the executing powers discussed on the Commission.

iii) Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and consequently retracting Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC a nd amending Directive 2000/60/EC of the European Parliament and of the Council.

iv) Regulation 1272/2008 (CLP) on classification, labelling and packaging of substances and mixtures.

v) Council Directive 67/548/EEC of 27 June 1967 on the estimation of laws, regulations and administrative requirements concerning to the classification, packaging and labelling of hazardous substances.

vi) Regulation (EC) No 166/2006 of the European Parliament and of the Council of 18 January 2006 relating to the establishment of a European Pollutant Release and Transfer Register and amending Council Directives 91/689/EEC and 96/61/EC

Self-assessment 2:

i) What is aquatic microbiology?

_____

_____

_____

_____

ii) Which microorganisms are present in fresh and marine water bodies?

_____

_____

_____

_____

iii) Explain in short about Littoral zone.

_____

_____

_____

_____

_____

iv) What do you know about Limnitic zone? Explain in short

_____

_____

_____

_____

v) Explain in brief about Benthic zone.

_____

_____

_____

_____

_____

vi) Salt loving bacteria are also known by which name?

_____

_____

_____

_____

4.6 Toxicity

There are two types of toxicity, i.e., direct and indirect toxicity. In direct toxicity, a toxicant takes action at the place of act in or on the organism. Indirect toxicity happens due to the alteration in the physical, chemical, or biological environment.

4.6.1 Factors which Influence Overall Toxicity in the Environment

Factors which Influence overall toxicity may be depicted as follows:

4.6.1.1. Factors Correlated to Chemical

Physico-chemical properties: Molecular structure, solubility, ionization, vapor pressure, rate constant for hydrolysis, photolysis, evaporation, sorption and partition coefficients and functional group influence the toxicological and pharmacological properties of the compound.

The non-polar (lipophilic) compounds are highly soluble in lipids and other organic solvents in comparison to polar (hydrophilic) compounds. Thus, lipophilic compounds can penetrate the lipoprotein layers and create their possible effects easily.

Concentration: The lower concentration of the chemical cause mild effects whereas high concentration may cause serious and long lasting effects.

Selective Toxicity: The chemicals are selective for their effects, i.e. all kind of chemicals can't affect all kind of organisms. Selective toxicity results from biological diversity and variations in the response of living cells and tissues to different chemicals.

Chemical Interaction: Occasionally organisms are exposed to not only to one chemical, but to two or three chemicals at the same time. These chemicals may interact with each other and create large toxicological impact.

The interaction of one chemical with another may have no effect, or increase, or decrease their toxicity.

4.6.1.2. Factors Related to Exposure

Any toxic material cannot effect, if not comes in the direct contact of the organism. In toxicity studies the mainly considerable factors associated to exposure are –

(i) The concentration of the chemical

(ii) The kind, duration and frequency of exposure, and

(iii) Routes of exposure

Aquatic animals come in contact to chemicals and wastes found in water, deposit and food stuff. Organisms get water soluble toxicants more easily in comparison to water insoluble chemicals.

4.6.1.3. Factor Related to Organism:

Toxicity of a chemical varies from organism to organism. The toxicity of a chemical shows a discrepancy according to the size, life history stage, age, sex and health of the organism.

4.6.1.4 Factors Related to Environment:

The abiotic and biotic factors of the environment can alter the toxicity of chemicals.

The temperature of water significantly affects the toxicity of xenobiotic chemicals. As the temperature of water raises, the solubility of many substances increases, the chemical structure of some toxicants modifies and the dissolved oxygen content of water becomes less. Though, some pesticides may be more toxic at higher temperatures whereas some may be poisonous at low temperatures.

The salinity and hardness of water also influence toxicity. The toxicity of xenobiotics may be increased with a significant decrease in the salinity of the surrounding. Though, the total hardness of water has slight effect on the toxicity of most of the chemicals except metals.

Several heavy metals are more toxic to aquatic biota, in very soft water than hard water.

4.7 Aquatic toxicology

Aquatic toxicology is the study of the effects of man-made chemicals and other artificial and natural materials and actions on aquatic organisms at diverse levels of group, from sub cellular throughout individual organisms to communities and ecosystems. Aquatic toxicology is a multifaceted field which assimilates toxicology, aquatic ecology and aquatic chemistry.

Aquatic toxicology consists of freshwater and marine water ecosystems. Regular tests consist of standardized acute and chronic toxicity tests lasting 24–96 hours (acute test) to 7 days or more (chronic tests). Endpoints of these tests may be survival, growth, reproduction that is considered at each concentration in a gradient, all along with a control test. Typically using selected organisms with ecologically significant sensitivity to toxicants and well-established literature surroundings. These organisms can be effortlessly obtained or cultured in lab and are simple to handle.

Testing Organisms and Toxicity Testing

Types of Organism	**Organism**
Invertebrate	Daphnia magna, Crayfish, Mayflies, Planaria
Aquatic Vertebrate	Rainbow Trout, Goldfish, Catfish
Algae	Chlamydomonas, Microcystis
Mammals	Rats, Mice
Avian Species	Ring Necked Pheasant

Source: Pollution Science, Academic Press, San Diego, CA, 1996.

4.8 Aquatic Toxicity Tests

Aquatic toxicology tests are also called assays. These toxicity tests make available qualitative and quantitative figures on undesirable (poisonous) effects of a toxicant on aquatic organisms.

Toxicity tests can be used to evaluate the probable harm to an aquatic environment and make available a record which can be used to review the hazard related with in a circumstances for a particular toxicant. Aquatic toxicology tests can be executed in the field or in laboratory. Field experiments usually refer to the coverage of many species and laboratory experiments to single species.

Various toxicity tests may be executed on different test species. These species may be different in their receptiveness to chemicals, metabolic rate, excretion rate, genetic factors, dietary factors, age, sex, health and stress level of the organism. General standard test species are the fathead minnow (Pimephales promelas), daphnids (Daphnia magna, D. pulex, D. pulicaria, Ceriodaphnia dubia), midge (Chironomus tentans, C. ruparius), rainbow trout (Oncorhynchus mykiss), sheepshead minnow (Cyprinodon variegatu), zebra fish (Danio rerio), mysids (Mysidopsis), oyster (Crassotreas), scud (Hyalalla Azteca), grass shrimp (Palaemonetes pugio) and mussels (Mytilus galloprovincialis). These species are usually chosen on the basis of accessibility, viability, frivolous, and ecological importance, past successful use, and dogmatic use.

A range of adequate consistent test methods are available. A few of the more extensively acknowledged agencies to publish methods are: The American Public Health Association, US Environmental Protection Agency (EPA), ASTM International, International Organization for Standardization, Environment and Climate Change Canada, and Organization for Economic Co-operation and Development. Standardized tests recommend the aptitude to evaluate results between laboratories.

Various types of toxicity tests are extensively accepted in the scientific literature and regulatory agencies. These tests depend on several factors: Particular regulatory agency accomplish the test, resources available, physical and chemical characteristics of the environment, type of toxicant, test species available, laboratory vs. field testing, end-point

assortment, and time and resources available to carry out the assays are some of the mainly frequently persuade factors on test design.

4.9 Exposure Systems

These are four common techniques for exposing the controls and test organisms with treated and diluted water or the test solutions.

Static: A static test rendered the organism in immobile water. The toxicant is added to the water in order to get the accurate concentrations to be tested. The control and test organisms are positioned in the test solutions. The water is untouched for the total period of test.

Recirculation: This test is similar to the static test, with the exception of that the test solutions are propelled through an apparatus (i.e. filter) to sustain quality of water, but the concentration of the toxicant in water is not reduced. The water is circulated through the test chamber constantly, similar to an aerated fish tank. This type of test is expensive and it is unclear whether or not the filter or aerator has an effect on the toxicant.

Renewal: This test is also done in still water. Though, in this test the test solution is renewed periodically (constant intervals) by relocating the organism to a fresh test chamber with the equal concentration of toxicant.

Flow-through: In this test the organism is rendered to the toxicant with a flow into the test chambers followed by out of the test chambers. The flow may either be alternating or continuous. A stock solution of the accurate concentrations of contaminant necessarily to be prepared earlier. Proper proportions of water and contaminant will be mixed by controlling the flow and volume of the test solution by pumps or diluters.

4.10 Types of Tests

The different types of tests are as follows-

Acute Tests: These tests are short-term exposure tests (hours or days). Lethality is taken as an endpoint in these tests. In severe contact, the higher doses of the toxicant are given to the organism in a single or in multiple trial in a small period of time. These generally generate instant effects, as according to the absorption time of the toxicant. These tests are commonly carried out on organisms all through a particular time period of the life cycle of

the organism. These tests are regarded as fractional life cycle tests. If death in the control sample is greater than 10%, these tests are not appropriate. Results are reported in EC50, or concentration that will influence fifty percent of the sample size.

Chronic Tests: These are long-term tests (weeks, months years), relative to the life span (>10% of life span) of the test organism. These tests usually use sub-lethal endpoints. Organisms come into contact with low and uninterrupted doses of a toxicant. Chronic exposures may stimulate effects to acute exposure, but effects may build up gradually. Chronic tests are usually taken as complete life cycle tests. These cover up a whole generation time or reproductive life cycle ("egg to egg"). Chronic tests are not taken as suitable if death in the control sample is greater than 20%. These results are generally reported in NOECs (No observed effects level) and LOECs (Lowest observed effects level).

Early Life Stage Tests: These tests are considered as sub chronic contact that is less than a whole reproductive life cycle. These consist of exposure all through near the beginning, receptive phase of life an organism. These tests are also called as critical life stage, embryo-larval, or egg-fry tests. Early life stage tests are not taken as applicable if death in the control sample is greater than 30%.

Short-Term Sub Lethal Tests: These tests are used to estimate the toxicity of effluents to aquatic organisms. These methods are developed by the EPA. These focused only on the most susceptible phase of life. Endpoints for this test consist of altered growth, reproduction and survival. NOECs, LOECs and EC50s are reported in these tests.

Bioaccumulation Tests: These are toxicity tests that can be used for hydrophobic chemicals, might be piled up in the fatty tissue of aquatic organisms. Toxicants having low solubility in water usually might be accumulated in the fatty tissue due to the high lipid content in this tissue. The accumulation of these toxicants inside the organism might be go ahead to cumulative toxicity. Bioaccumulation tests use bio concentration factors (BCF) to calculate concentrations of hydrophobic contaminants in organisms. The BCF is the ratio of the average concentration of test chemical stored in the tissue of the test organism (under steady state conditions) to the average calculated concentration in the water.

Freshwater Tests and saltwater Tests: Regulatory agencies have made different standard methods. Though, these tests usually contain a control (negative and/or positive), a geometric dilution series or other suitable logarithmic dilution series, test chambers, equal numbers of replicates and a test organism. Precise exposure time and test period depend on type of test (acute vs. chronic) and organism. Temperature, water quality parameters and light depend on regulatory requirements and organism type.

Self-assessment 3

1. Write the names of two types of toxicity.

_____
_____
_____
_____
_____

2. Write the factors which Influence overall toxicity.

_____
_____
_____
_____
_____

3. What is Aquatic Toxicology?

_____
_____
_____
_____
_____

4. Write a short note on Aquatic Toxicity Tests.

_____
_____
_____
_____
_____

5. Write the four common techniques for exposing the controls and test organisms with treated and diluted water or the test solutions.

_____
_____
_____
_____
_____

6. Write the names of different types of tests.

_____

_____

_____

_____

_____

Summary

- Aquatic microbiology is the study of microbes of aquatic systems.
- It includes microscopic plants, animals, bacteria, viruses and fungi and their behaviour, relation with other organisms in aquatic environment.
- Bacteria, virus and fungi are found throughout the aquatic environment.
- Bacteria, virus and fungi can be found in fresh and marine ecosystems.
- Marine ecosystem is the biggest of the entire ecosystems.
- It covers up roughly 71% of the surface of the earth.
- Marine ecosystem includes about 97% of the water of the planet.
- Freshwater ecosystems cover up 0.78% of the Earth's surface. It occupies 0.009% of its whole water.
- There are three fundamental types of freshwater ecosystems:
- Lentic: This type of ecosystem is of slow moving water, including pools, ponds, and lakes.
- Lotic: It is of faster moving water, includes streams and rivers.
- Wetlands: It is of areas where the soil is saturated or flooded for at least part of the time.
- An ecosystem has two components: Biotic and Abiotic factors.
- Biotic factors contains biological interactions among various species of plants and animals.
- Abiotic contains environmental factors such as substrate type, water deepness, nutrient levels, temperature, moisture, salinity and flow.

- Ecoxicology is the study of the effect of toxic chemicals on biological organisms particularly at the population, society, ecosystem, and biosphere levels.
- The term "ecotoxicology" was contrived by René Truhaut in 1969.
- Aquatic Toxicants are the chemicals characterize a significant harm to living organisms and human health all the way through aquatic contact.
- The harmful effects of aquatic toxicants may be damage to the reproductive, immune, endocrine and/or nervous systems, cancer and even death.
- Aquatic toxicology is the study of the effects of man-made chemicals and other artificial and natural materials and actions on aquatic organisms at diverse levels of group, from sub cellular throughout individual organisms to communities and ecosystems.
- Aquatic toxicology is a multifaceted field which assimilates toxicology, aquatic ecology and aquatic chemistry.

Terminal Questions

1. Explain in detail of an Aquatic Ecosystem.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

2. Explain in detail about the components of an Aquatic Ecosystem.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

3. Explain in detail about the Ecotoxicology. Explain regular environmental toxicants.

4. Explain in detail the aquatic toxicants.

5. What is toxicity? Explain the factors which influence overall toxicity in the environment.

6. What is aquatic toxicology? Explain testing organisms and toxicity testing.

7. Write in detail about the aquatic toxicity tests.

_____
_____
_____
_____
_____
_____

Answers

Self-assessment 1

Refer to 4.3

Refer to 4.3.1

Refer to 4.3

Refer to 4.3.1

Refer to 4.3.1

Refer to 4.3.1

Refer to 4.3.1

Self-assessment 2

Refer to 4.4

Refer to 4.4

Refer to 4.4

Refer to 4.4

Refer to 4.4

Refer to 4.4

Self-assessment 3

Refer to 4.6

Refer to 4.6.1

Refer to 4.7

Refer to 4.8

Refer to 4.9

Refer to 4.10

Terminal Questions

Refer to 4.3

Refer to 4.3.2

Refer to 4.5

Refer to 4.5.3

Refer to 4.6

Refer to 4.7

Refer to 4.8, 4.9, and 4.10

Reference

1. Alexander, David E. (1 May 1999). Encyclopedia of Environmental Science. Springer. ISBN 0-412-74050-8.
2. "University of California Museum of Paleontology: The Marine Biome". Retrieved 27 September 2018.
3. Alexander, David E. (1 May 1999). Encyclopedia of Environmental Science. Springer. ISBN 0-412-74050-8.
4. United States Environmental Protection Agency (2 March 2006). "Marine Ecosystems". Retrieved 25 August2006.
5. Daily, Gretchen C. (1 February 1997). Nature's Services. Island Press. ISBN 1-55963-476-6.
6. Vaccari, David A. (8 November 2005). Environmental Biology for Engineers and Scientists. Wiley-Interscience. ISBN 0-471-74178-7.
7. Clegg, J. (1986). Observer's Book of Pond Life. Frederick Warne, London. 460 p.
8. Clegg, J. (1986). Observer's Book of Pond Life. Frederick Warne, London. 460 p. p.160-163.
9. Keddy, Paul A. (2010). Wetland Ecology. Principles and Conservation. Cambridge University Press. p. 497. ISBN 978-0-521-51940-3.
10. Keddy, P.A., D. Campbell, T. McFalls, G. Shaffer, R. Moreau, C. Dranguet, and R. Heleniak. 2007. The wetlands of lakes Pontchartrain and Maurepas: past, present and future. Environmental Reviews 15: 1- 35.
11. Gastescu, P. (1993). The Danube Delta: geographical characteristics and ecological recovery. Earth and Environmental Science, 29, 57–67.
12. https://www.encyclopedia.com/places/oceans-continents-and-polar-regions/oceans-and-continents/aquatic-microbiology
13. Loeb, Stanford L. (24 January 1994). Biological Monitoring of Aquatic Systems. CRC Press. ISBN 0-87371-910-7
14. Vallentyne, J. R. (1974). The Algal Bowl: Lakes and Man, Miscellaneous Special Publication No. 22. Ottawa, ON: Department of the Environment, Fisheries and Marine Service.
15. Moss, B. (1983). The Norfolk Broadland: experiments in the restoration of a complex wetland. Biological Reviews of the Cambridge Philosophical Society, 58, 521–561.
16. Keddy, P. A., Campbell, D., McFalls T., Shaffer, G., Moreau, R., Dranguet, C., and Heleniak, R. (2007). The wetlands of lakes Pontchartrain and Maurepas: past, present and future. Environmental Reviews, 15, 1–35.
17. Graham, J. B. (1997). Air Breathing Fishes. San Diego, CA: Academic Press.
18. Sculthorpe, C. D. (1967). The Biology of Aquatic Vascular Plants. Reprinted 1985 Edward Arnold, by London.
19. Manahan, Stanley E. (1 January 2005). Environmental Chemistry. CRC Press. ISBN 1-56670-633-5.
20. Smith, V. H. (1982). The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. Limnology and Oceanography, 27, 1101–12.

21. Smith, V. H. (1983). Low nitrogen to phosphorus ratios favor dominance by bluegreen algae in lake phytoplankton. Science, 221, 669–71.

22. Turner, R. E. and Rabelais, N. N. (2003). Linking landscape and water quality in the Mississippi River Basin for 200 years. BioScience, 53, 563–72.

23. Silliman, B. R., Grosholz, E. D., and Bertness, M. D. (eds.) (2009). Human Impacts on Salt Marshes: A Global Perspective. Berkeley, CA: University of California Press.

24. Chapman, J.L.; Reiss, M.J. (10 December 1998). Ecology. Cambridge University Press. ISBN 0-521-58802-2.

25. Barange M, Field JG, Harris RP, Eileen E, Hofmann EE, Perry RI and Werner F (2010) Marine Ecosystems and Global ChangeOxford University Press. ISBN 978-0-19-955802-5

26. Boyd IL, Wanless S and Camphuysen CJ (2006) Top predators in marine ecosystems: their role in monitoring and management Volume 12 of Conservation biology series. Cambridge University Press. ISBN 978-0-521-84773-5

27. Christensen V and Pauly D (eds.) (1993) Trophic models of aquatic ecosystems The WorldFish Center, issue 26 of ICLARM Technical Reports, volume 26 of ICLARM conference proceedings. ISBN 9789711022846.

28. Davenport J (2008) Challenges to Marine Ecosystems: Proceedings of the 41st European Marine Biology SymposiumVolume 202 of Developments in hydrobiology. ISBN 978-1-4020-8807-0

29. Levner E, Linkov I and Proth J (2005) Strategic management of marine ecosystems Springer. Volume 50 of NATO Science Series IV. ISBN 978-1-4020-3158-8

30. Mann KH and Lazier JRN (2006) Dynamics of marine ecosystems: biological-physical interactions in the oceansWiley-Blackwell. ISBN 978-1-4051-1118-8

31. Moustakas A and Karakassis I (2005) "How diverse is aquatic biodiversity research?" Aquatic Ecology, 39: 367-375.

32. National Research Council (US) (1996) Freshwater ecosystems: revitalizing educational programs in limnology National Academy Press. ISBN 0-309-05443-5

33. *Rand, Gary M.; Petrocelli, Sam R. (1985). Fundamentals of aquatic toxicology: Methods and applications. Washington: Hemisphere Publishing.* ISBN 978-0-89116-382-4.

34. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol 1 *(Report). Washington, DC: U.S. Environmental Protection Agency (EPA). September 2001. EPA 821-B-01-004.*

35. "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition". *EPA. October 2002. EPA 821-R-02-012.*

36. "Water Quality Standards History" EPA. Received 2012-06-06 Archived 2012-06-28 at the Wayback Machine

37. *Calow, Peter P (2009). Handbook of Ecotoxicology. John Wiley & Sons. p. 900.* ISBN 978-1444313505.

38. *Liu, Fu-Jun; Wang, Jia-Sheng; Theodorakis, Chris W. (May 2006). "Thyrotoxicity of Sodium Arsenate, Sodium Perchlorate, and Their Mixture in ZebrafishDanio rerio". Environmental Science & Technology. 40 (10): 3429–3436.* doi:10.1021/es052538g. ISSN 0013-936X.

39. *Vidal-Liñán, Leticia; Bellas, Juan; Campillo, Juan Antonio; Beiras, Ricardo (January 2010). "Integrated use of antioxidant enzymes in mussels, Mytilus galloprovincialis, for monitoring pollution in highly productive coastal areas of Galicia (NW Spain)". Chemosphere. 78 (3): 265–272.* doi:10.1016/j.chemosphere.2009.10.060. PMID 19954813.
40. "Whole Effluent Toxicity (WET)". *National Pollutant Discharge Elimination System (NPDES). EPA. 2017-10-10.*
41. "Whole Effluent Toxicity Methods". *EPA. 2018-04-19.*
42. "About ASTM International". *West Conshohocken, Pennsylvania*. Retrieved 2018-12-24.
43. *Baird, Rodger B.; Clesceri, Leonore S.; Eaton, Andrew D.; et al., eds. (2012).* Standard Methods for the Examination of Water and Wastewater *(22nd ed.). Washington, DC: American Public Health Association.* ISBN 978-0875530130.
44. "ECOTOX Knowledgebase" EPA. Accessed 2018-12-13.
45. "Society of Environmental Toxicology and Chemistry". *Pensacola, Florida*. Retrieved 2018-12-24.
46. "About the Organisation for Economic Co-operation and Development". *Paris, France*. Retrieved 2018-12-24.
47. "Environment and Climate Change Canada's Mandate". *Ottawa, Ontario. 2018-12-10.*
48. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses *(Report). EPA. 1985. PB85-227049.*
49. "Sediment Quality Guidelines developed for the National Status and Trends Program" Archived June 12, 2013, at the Wayback Machine National Status & Trends, 1999
50. "SQuiRT" National Oceanic and Atmospheric Administration, 2008
51. http://www.biologydiscussion.com/environment/factors-which-affect-overall-toxicity-in-the-environment/4902
52. https://risctox.istas.net/en/index.asp?idpagina=614

Unit 5: Drinking Water treatment

Unit Structure

5.0 Learning Objectives

After studying this unit you would be able to:

- understand the types of microbial contaminants
- know about the indicator organisms
- discuss the current microbiological techniques used for maintaining potable water quality
- discuss the isolation of different bacteria according to MPN index

5.1 Introduction

We know that water is elixir on earth and essential to life, but many people do not have access to clean and safe drinking water and many die due to water-borne bacterial infections. The contaminants in drinking water cause serious health hazards which include nausea, lung irritation, skin rash, vomiting, dizziness and even death. Microbiological analysis of water is mainly based on various types of bacterial study.

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries. For example, in pre-

Christian times the storage of drinking water in jugs made of metal was practiced. Now, the anti-bacterial effect of some metals is also known.

Chemicals such as chlorine or chlorine derivatives has been used for killing bacteria such as *Escherichia coli* in water, since the early decades of the twentieth century. Other bacteria-killing treatments that are increasingly becoming popular include the use of a gas called ozone and the disabling of the microbe's genetic material by the use of ultraviolet light. Microbes can also be excluded form water by using the filter. Modern filters have tiny holes in them so that even particles as miniscule as viruses can be trapped.

An important aspect of drinking water microbiology is the testing of the water to ensure that it is safe to drink. Water quality testing can be done in several ways. One popular test measures the turbidity of the water. It is used for the indication of the amount of suspended material in the water. The presence of particles even as small as bacteria and viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating.

The testing for microbes that cause disease (i.e., *Salmonella typhymurium* and *Vibrio cholerae*), can be expensive and if the bacteria are present in low numbers, they may escape detection. *Escherichia coli* has been used as an indicator of faecal pollution for decades. The bacterium is present in the intestinal tract in huge numbers. The presence of the bacterium in water is indicative of recent faecal pollution.

It is well known that water is a natural resource and it is necessary for sustaining life in this universe. The quality of drinking water should be free from health risks. It has been noted that water is contaminated by various microbial and non-microbial contaminants now. According to United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) *E. coli* should be nil in 100 mL water samples (USEPA, 1990; WHO,1994). No sample should contain more than 10 coliform organisms per 100 mL. Some parameters of Indian standards for drinking water are given in Table 01.

Table 01: Drinking Water Standards of BIS (IS:10500:1991).

S.No.	Parameters Essential Characteristics	Desirable Limits mg/L	Permissible Limits mg/L

1	Colour	Hazen unit	5 25
2	Odour	Unobjectionable	-
3	Taste	Agreeable	-
4	Turbidity (NTU)	5	10
5	Ph	6.5-8.5	No relaxation
6	Total hardness, $CaCO_3$	300	600
7	Iron (Fe)	0.3	1.0
8	Chloride (Cl)	250	1000
9	Residual free chlorine	0.2	-
10	Fluoride (F)	1.0	1.5

NTU = Nephelometric Turbidity Unit

Source: BIS (IS: 10500:1991)

5.2. Microbes in drinking water

Safe drinking water is the need of every human being. Water-borne infections are common in developing countries, access to clean water is not possible for everybody. According to WHO, the mortality of water associated diseases exceeds 5 million people per year. Among these, more than 50 per cent are microbial intestinal infections, cholera caused by presence of *V. cholerae* in drinking water has been observed to be a major disease. The greatest microbial risks are associated with ingestion of water that is contaminated with human or animal faeces. (WHO, 2019)

Identification of water sources can help in identifying the ones particularly at risk for protozoan contamination. In spatiotemporal characteristics investigation, municipal water is found to be a potential source of faecal contaminants and acts as a predominant vehicle for transmission of *S. typhi* and *S. paratyphi*. Highly localized human waste has been detected as a major contributor to poor water quality. Different diseases caused a by a variety of microorganisms are given in Table 02. **(Cabral, 2010)**

Table 02: Showing Disease Causal Organisms.

S.No.	Disease	Causal Organisms
1	Cholera	*Vibrio cholerae*, serovarieties O1 and O139
2	Gastroenteritis	*Vibrio parahaemolyticus*

3	Typhoid fever and other serious salmonellosis	*Salmonella enterica* sub sp. enteric serovar paratyphi, typhi
4	Bacillary dysentery or shigellosis	*Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*
5	Acute diarrhoeas and gastroenteritis	*Escherichia coli*, particularly serotypes such as O148, O157 and O124

Source: Cabral (2010)

Many types of microorganisms can get the support from water for their growth. The presence of other disease causing microbes in water is unhealthy and life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli* , *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if faeces enters the water. Contamination of drinking water with a type of *Escherichia coli* known as O157:H7, can be fatal. (Sehgal et al.,2008)

The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples are rotavirus, enteroviruses, and coxsackievirus.

5.3. Indicator Organisms

Faecal indicators are a group of organisms that demonstrate the efficacy of a process. The most widely used indicators are coliforms (total coliforms), faecal or thermotolerant coliforms, E. *coli*, *Enterococci* (faecal *streptococci* or intestinal *enterococci*) and bacteriophages. There are different enteric microorganisms that are known to infect humans. Some of them are as follows:

1 Total Coliform: The term "total coliform" refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermotolerant coliforms and bacteria of faecal origin, as well as some bacteria that may be isolated from environmental sources.

The total group includes faecal coliform bacteria such as *E. coli* as well as other types of coliform bacteria. Total coliforms do not necessarily indicate recent water contamination by faecal waste. However, the presence or absence of these bacteria in treated water is often used to determine whether water disinfection is working properly. Among the total coliform, *Pseudomonas aeruginosa*, *Shewanella putrefaciens*, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Proteus mirabilis* are also reported. (WHO 2008).

2 Coliforms: Coliform bacteria are facultative anaerobes, Gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with acid production in 24 to 48 h at 36ºC. Coliforms belong to the family Enterobacteriaceae and include *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter*, *Kluyvera*, *Leclercia* genera, and some members of the genus *Serratia*. These bacteria have been classically used as indicators of faecal contamination of water. Coliform bacteria, traditionally termed the total coliform group, have been the primary standard for potable water in most of the world. (WHO 2008).

3 Faecal Coliforms : These bacteria conform to all the criteria used to define total coliforms. Coliform bacteria are considered "indicator organisms"; their presence warns of the potential presence of disease causing organisms and should alert the person responsible for the water supply to take precautionary action. (WHO 2008).

4 Escherichia Coli : *E. coli* is a member of faecal coliform group and is enzymatically distinguished by the lack of urease and presence of b-glucuronidase. *E. coli* is the faecal indicator of choice used in WHO Guidelines for Drinking-water quality (WHO 2008).

Although it has long been known that *E. coli* can cause disease in humans, the bacteria naturally occur in the lower part of the gut of warm-blooded animals. Its role as an enteric pathogen has been strengthened with the discovery of *E. coli* O157:H7 associated with haemorrhagic enteritis and haemolytic uremic syndrome that was responsible for producing several drinking water outbreaks. (Sehgal et al.,2008)

5 Faecal Streptococci, Enterococci or Intestinal Enterococci: This group of microorganisms has received widespread acceptance as useful indicators of microbiological water quality.

Presence of faecal coliforms and faecal Streptococci in drinking water indicates contaminated water sources, inadequate treatment or post-treatment contamination of drinking water.

The presence of faecal coliform in drinking water is a strong indication of recent sewage or animal waste contamination. The presence of heterotrophic bacteria in drinking water is not an indication that the water presents a health risk. Rather, no specific significance or health standards are associated with these non-pathogenic, non-coliform bacteria.

Total coliform bacteria are generally not harmful but some microbes in the wastes can cause short-term effects, such as diarrhoea, cramps, nausea, headaches, or other symptoms. Infants, young children, some of the elderly, and people with severely compromised immune systems may be more susceptible than the general public.

6 Sulphite-reducing clostridia (SRC): These are gram-positive, spore-forming, non-motile, strictly anaerobic rods that reduce sulphite to H2S.

7 Clostridium perfringens: C. perfringens is the appropriate microbial water indicator.

8 Bacteriophages (phages): Literally means of bacteriophage is bacteria eater. These are bacterial based viruses and are ubiquitous in the environment. These viruses kill the bacteria by entering in their body. All bacteriophages are composed of a nucleic acid molecule that is surrounded by a protein structure.

5.4. Bacteriological Analytical Techniques

Two techniques are commonly used to detect the presence of coliforms in water as:

1) The "multiple fermentation tube" or "most probable number" technique. In this method measured portions of a water sample are placed in test-tubes containing a culture medium. The tubes are then incubated for a standard time at a standard temperature.

2) In the second technique, a measured volume of sample is passed through a fine filter that retains bacteria. The filter is then placed on culture medium and incubated. This is called the "membrane filter" technique.

Features of the two techniques are compared in Table 03.

Table 03: Comparison of methods for analysis of coliform bacteria

S.No.	Multiple fermentation tube technique OR MPN Technique	Membrane filter technique
1	Slower: requires 48 hours for a positive	More rapid: quantitative results in or presumptive positive about 18 hours
2	More labour-intensive	Less labour-intensive
3	Requires more culture medium	Requires less culture medium
4	Requires more glassware	Requires less glassware
5	More sensitive	Less sensitive

6	Result obtained indirectly by statistical approximation (low precision)	Results obtained directly by colony count (high precision)
7	Not readily adaptable for use in the field	Readily adapted for use in the field
8	Applicable to all types of water	Not applicable to turbid waters
9	Consumables readily available in most countries	Cost of consumables is high in many countries

Source: ISO1990b, UNEPWHO, Water quality

5.5 Multiple fermentation tube technique Or MPN Technique (MPN Index)

The technique has been used for the analysis of drinking-water for many years with satisfactory results. It is the only procedure that can be used if water samples are very turbid or if semi-solids such as sediments or sludges are to be analysed. MPN index is an index of the number of coliform bacteria.

Principle: The technique conducted on five portions of each of three serial dilutions of a water sample. The individual portions are used to inoculate tubes of culture medium that are then incubated at a standard temperature for a standard period of time. The presence of coliforms is indicated by turbidity in the culture medium, by a pH change and/or by the presence of gas. The MPN index is determined by comparing the pattern of the number of tubes showing growth at each dilution with statistical tables. The tabulated value is reported as MPN per 100 ml of sample. The most common procedure is to process five aliquots of water from each of three consecutive 10-fold dilutions; for example, five aliquots of the sample itself, five of a 1/10 dilution of the sample and five of a 1/100 dilution. Aliquots may be 1-ml volumes, each added to 10 ml of single strength culture medium, or 10-ml volumes, each added to 10 ml of double-strength medium. The use of one of the following variants of the technique may help to reduce the cost of analysis:

• A smaller number of tubes is incubated at each dilution, for example three instead of five. A different table must then be used for the MPN determination. Some precision is lost, but using 9 tubes instead of 15 saves materials, space in the incubator, and the analyst's time.

• For samples of drinking water, one tube with 50 ml of sample and five tubes with 10 ml of sample are inoculated and incubated to obtain the MPN.

Culture media and buffered dilution water: Each part of the test requires a different type of medium. For example, when enumerating coliforms, lauryl tryptose (lactose) broth is used in the first (isolation or presumptive) part of the test. In the second (confirmation) part, brilliant green lactose bile (BGLB) broth is used to confirm total coliforms and E. coli medium to confirm faecal coliforms. Media can be made from primary ingredients but are also available in the following forms:

• Dehydrated powder, packaged in bulk (200 g or more), to be weighed out when the medium is prepared and dissolved in an appropriate volume of distilled water, dispensed to culture tubes and sterilised before use.

• Dehydrated powder, packaged in pre-weighed amounts suitable for making one batch of medium, to be dissolved in an appropriate volume of distilled water, dispensed to culture tubes and sterilised before use.

* Pre-weighed packages are easy to use and reduce the risk of error in making up a batch of medium. Large bottles containing dehydrated media must be tightly resealed after use to prevent spoilage. Media should be stored in a cool, dark, dry place.

After a medium has been prepared by dissolving the powder in distilled water, it should be distributed into culture tubes or bottles and sterilised. Batches of media should be tested before use, using a known positive and negative control organism. If the appropriate reactions are not observed, the media and the control organisms should be investigated and the tests repeated. Media should be used immediately but may be stored for several days provided that there is no risk of their becoming contaminated.

A stock solution of buffered dilution water is prepared by dissolving 34.0 g of potassium dihydrogen phosphate, KH_2PO_4, in 500 ml of distilled water. The pH is checked and, if necessary, adjusted to 7.2 by the addition of small quantities of 1 mol l-1 NaOH solution. Distilled water is added to bring the final volume to 1 litre. The buffered water is stored in a tightly stoppered bottle in the refrigerator. To prepare bottles of dilution water, 1.25 ml of stock solution is added to 1 litre of distilled water, mixed well and dispensed into dilution bottles in quantities that will provide, after sterilisation, 9 or 90 ml. The bottles are loosely

capped, placed in the autoclave, and sterilised for 20 minutes at 121 °C. After the bottles have been removed from the autoclave, the caps should be tightened and the bottles stored in a clean place until needed. Volumes of 0.1 and 0.01 ml of sample are obtained by addition of 1 ml of a 1/10 and 1/100 dilution, respectively, of the sample to 10 ml of single-strength culture medium.

Different culture medias as Isolation and Confirmatory have been shown for MPN in table 04.

Table 04: Culture media for most probable number (MPN) analyses

S.No.	Medium	Uses	Incubation temperature	Remarks
Isolation media				
	Lactose broth	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	Prepare single strength medium by diluting double strength medium with distilled water. Each tube or bottle should contain an inverted fermentation (Durham) tube
	MacConkey broth	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	
	Improved formate lactose glutamate medium	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	Available commercially in dehydrated form as Minerals Modified Glutamate Medium

	Lauryl tryptose (lactose) broth	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	
Confirmatory media				
	Brilliant green lactose bile broth	Total or thermotolerant coliforms (gas production)	44.5 ± 0.25 °C for thermotolerant coliforms	
	EC medium	Thermotolerant coliforms (indole production)	44.5 ± 0.25 °C for thermotolerant coliforms	Addition of 1 % (m/m) L- or DLtryptophan may improve performance of the medium
	Tryptone water	Thermotolerant coliforms (gas + indole production)	44.5 ± 0.25 °C for thermotolerant coliforms	
	Lauryl tryptose mannitol broth with tryptophan	Thermotolerant coliforms (gas + indole production)	44.5 ± 0.25 °C for thermotolerant coliforms	

Source: Adapted from ISO, 1990b

Apparatus: Different apparatus are used in MPN Techniques are as follows:

- Incubator(s) or water-baths capable of maintaining a temperature to within ± 0.5 ºC of 35 and 37 ºC and to within ± 0.25 ºC of 44 and 44.5 ºC. The choice of temperature depends on the indicator bacteria and the medium
- Autoclave for sterilising glassware and culture media. The size required depends on the volume of work to be undertaken. A capacity of 100-150 litres would be required for a medium-size laboratory undertaking work on a routine basis.
- Distillation apparatus, with storage capacity for at least 20 litres of distilled water.

- Laboratory balance, accuracy ± 0.05 g, with weighing scoop. This may be omitted if culture media and potassium dihydrogen phosphate are available in pre-weighed packages of the proper size.
- Racks for tubes and bottles of prepared culture media and dilution water. These must fit into the autoclave.
- Pipettes, reusable, glass, 10-ml capacity graduated in 0.1-ml divisions, and 1-ml capacity graduated in 0.01-ml divisions.
- Test-tubes, 20 × 150 mm for 10 ml of sample + 10 ml of culture medium, with metal slip-on caps.
- Bottles, with loose-fitting caps, calibrated at 50 and 100 ml, for 50 ml of sample + 50 ml of culture medium.
- Measuring cylinders, unbreakable plastic or glass, capacity 100, 250, 500 and 1,000 ml.
- Test-tube racks to hold tubes in incubator and during storage.
- Thermometer for checking calibration of incubator or water-bath.
- Refrigerator for storage of prepared culture media.
- Hot-air steriliser for sterilising pipettes.
- Bunsen burner or alcohol lamp.
- Durham tubes, 6 × 30 mm.
- Pipette cans for sterilising pipettes.
- Flasks for preparation of culture media.
- Wash-bottle.
- Pipette bulbs.
- Wire loops for inoculating media, and spare wire.
- Spatula.
- Container for used pipettes.
- Brushes for cleaning glassware (several sizes).

- Fire extinguisher and first-aid kit.
- Miscellaneous tools.
- Waste bin. Consumables
- Culture media: for example lauryl tryptose broth, brilliant green lactose bile (BGLB) broth, and E. coli medium.
- Disinfectant for cleaning laboratory surfaces and the pipette discard container.
- Detergent for cleaning glassware and equipment.
- Phosphate-buffered dilution water.
- Autoclave tape.

Procedure: The procedure described below is for five tubes at each of three sample dilutions and provides for confirmation of both total and thermotolerant (faecal) coliforms. If fewer tubes (e.g. three of each of three sample dilutions or one 50-ml and five 10-ml portions) are inoculated, the MPN index must be determined from tables specific to the combination of tubes and dilutions used.

1. Prepare the required number of tubes of culture medium. The volume and strength (single or double) of medium in the tubes will vary depending on the expected bacteriological density in the water and the dilution series planned. For most surface waters, 10 ml volumes of single-strength medium are appropriate.

2. Select and prepare a range of sample dilutions; these will normally be suggested by experience. Recommended dilutions for use when there is no experience with samples from that station are given in **Table 05.**

Table 05: Table showing typical sample volumes and number of tubes for multiple fermentation tube analysis.

Sample type	**Sample volume (ml)**				
	50	10	1	0.1	0.01
Treated drinking-water	1	5			
Partially treated drinking-water		5	5	5	
Recreational water		5	5	5	
Protected-source water		5	5	5	

Surface water			5	5	5

To prepare a 1/10 dilution series, mix the sample bottle well. Pipette 10 ml of sample into a dilution bottle containing 90 ml of phosphate-buffered dilution water. To prepare a 1/100 dilution, mix the 1/10 dilution bottle well and pipette 10 ml of its contents into a bottle containing 90 ml of dilution water. Subsequent dilutions are made in a similar way. Alternatively, 1 ml of sample may be added to a bottle containing 9 ml of dilution water.

3. Pipette the appropriate volumes of sample and diluted sample into the tubes of medium, as shown in **Figure 01a**.

4. Label the tubes with the sample reference number, the dilution and the volume of sample (or dilution) added to the tube. Shake gently to mix the sample with the medium. Place the rack in an incubator or water-bath for 48 hours at 35 ± 0.5 ºC or 37 ± 0.5 ºC.

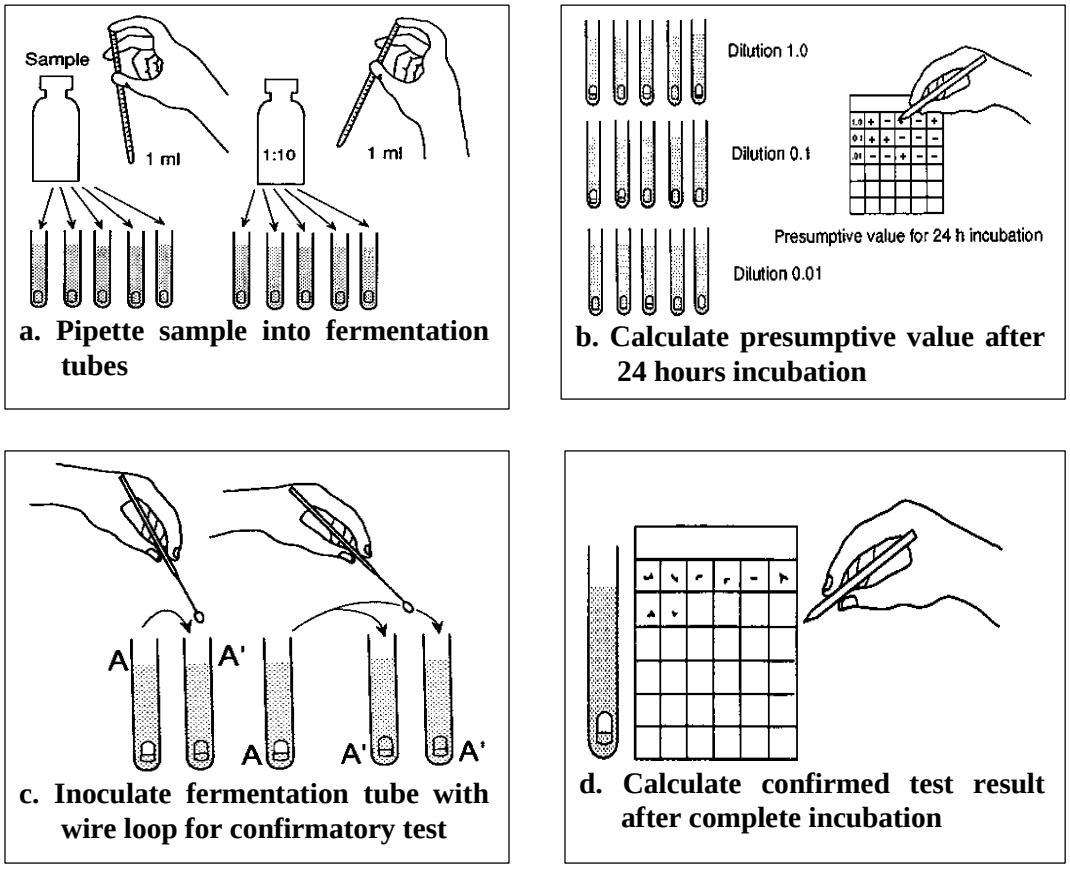
5. After 18 or 24 hours, note which tubes show growth. The reactions are listed in Table 06. Tubes that show turbidity and gas production, or a colour change indicating the production of acid (if the medium contains a pH indicator), are regarded as positive. Record the number of positive tubes at each dilution, as shown in **Figure 01,b**. Return the tubes to the incubator and re-examine after a total of 48 hours of incubation. Continue with the next step of the procedure.

6. Prepare the required number of tubes of confirmation culture medium (BGLB broth for total coliforms and *E. coli* medium for faecal coliforms). Using a sterile wire loop, transfer inocula from positive tubes into the confirmation medium, as shown in **Figure 01,c**. Sterilise the loop between successive transfers by heating in a flame until it is red hot. Allow it to cool before use. If confirmation of both total and faecal coliforms is required, a BGLB and an *E. coli* medium tube should be inoculated from each presumptive positive. Label these tubes carefully with the same code used in the presumptive test and incubate them for 48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms (BGLB broth) or for 24 hours at 44 ± 0.5 °C for faecal coliforms (*E. coli* medium).

7. After the prescribed incubation time, note which tubes show growth with the production of gas, and record the number of positives for each sample dilution as shown in **Figure 01,d**.

8. Compare the pattern of positive results with a most probable number table such as one of those given in Tables 05, 06, 07, 08 and 09.

Figure 01: Steps in the multiple fermentation tube technique



a. Pipette sample into fermentation tubes

b. Calculate presumptive value after 24 hours incubation

c. Inoculate fermentation tube with wire loop for confirmatory test

d. Calculate confirmed test result after complete incubation

Source: Adapted from ISO, 1990b

Table 06: Showing reactions following analysis by the MPN method

Medium	Reactions	
	Total coliforms at 35 or 37 °C	Thermotolerant coliforms at 44 or 44.5 °C
Isolation media		
Lactose broth	Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium	Same as total coliforms at 35 or 37°C
MacConkey broth	Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium	Same as total coliforms at 35 or 37°C
Improved formate lactose glutamate medium	Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium	Same as total coliforms at 35 or 37°C
Lauryl tryptose (lactose) broth	Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium	Same as total coliforms at 35 or 37

		°C
Confirmatory media		
Brilliant green lactose bile broth	Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium	Same as total coliforms at 35 or 37°C
EC medium	Gas visible in the inverted fermentation (Durham) tube plus turbidity of the medium	Same as total coliforms at 35 or 37°C
Tryptone water		Add KOVACS' reagent to tube; a red colour denotes presence of indole
Lauryl tryptose mannitol broth with tryptophan		Allows detection of gas + indole production in same tube

Source: Adapted from ISO, 1990b

Table 07: Showing MPN index and 95 per cent confidence limits for various combinations of positive results when five tubes are used per dilution (10 ml, 1.0 ml, 0.1 ml portions of sample)

Combination of positives	MPN index per 100 ml	95 % confidence limits		Combination of positives	MPN index per 100 ml	95 % confidence limits	
		Upper	Lower			Upper	Lower
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1	33	15	77
				4-4-0	34	16	80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1	50	20	150
				5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360

3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1,300
4-1-0	17	7.0	46	5-5-2	500	200	2,000
4-1-1	21	9.0	55	5-5-3	900	300	2,900
4-1-2	26	12.0	63	5-5-4	1,600	600	5,300
				5-5-5	>1,600	-	-

Source: After APHA, 1992

Table 08: Showing MPN index for various combinations of positive results when three tubes are used per dilution (10 ml, 1.0 ml and 0.1 ml portions of sample)

Number of tubes giving a positive reaction from			MPN
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each	
0	0	0	<3
0	0	1	3
0	1	0	3
1	0	0	4
1	0	1	7
1	1	0	7
1	1	1	11
1	2	0	11
2	0	0	9
2	0	1	14

Source: After WHO, 1985

Table 09: Showing MPN index and 95 per cent confidence limits for various combinations of positive results for a set of one 50 ml and five 10 ml portions of sample

Number of tubes giving positive reaction		MPN Index per 100 ml	95 % confidence limits	
1 × 50 ml	5 × 10 ml		Lower	Upper
0	0	<1		
0	1	1	0.5	4
0	2	2	0.5	6
0	3	4	0.5	11
0	4	5	1	13
0	5	7	2	17
1	0	2	0.5	6
1	1	3	0.5	9
1	2	6	1	15
1	3	9	2	21
1	4	16	4	40
1	5	>18		

Source: After Department of Health and Social Security, 1982

5.5. Water quality standards for drinking water

International drinking water standards like WHO Drinking Water Standards, EPA Drinking Water Standards, Indian Drinking Water Standards BIS-10500 are set by Government Organisations. Shown below is a **Table 10** of the Indian Water Quality Standards.

INDIAN STANDARD SPECIFICATIONS FOR DRINKING WATER IS: 10500

S.NO.	Parameter	Requirement desirable Limit	Remarks
1.	Colour	5	May be extended up to 50 if toxic substances are suspected
2.	Turbidity	10	May be relaxed up to 25 in the absence of alternate
3.	pH	6.5 to 8.5	May be relaxed up to 9.2 in the absence
4.	Total Hardness	300	May be extended up to 600
5.	Calcium as Ca	75	May be extended up to 200
6.	Magnesium as Mg	30	May be extended up to 100
7.	Copper as Cu	0.05	May be relaxed up to 1.5
8.	Iron	0.3	May be extended up to 1
9.	Manganese	0.1	May be extended up to 0.5
10.	Chlorides	250	May be extended up to 1000
11.	Sulphates	150	May be extended up to 400
12.	Nitrates	45	No relaxation
13.	Fluoride	0.6 to 1.2	If the limit is below 0.6 water should be rejected, Max. Limit is extended to 1.5
14.	Phenols	0.001	May be relaxed up to 0.002
15.	Mercury	0.001	No relaxation
16.	Cadmium	0.01	No relaxation
17.	Selenium	0.01	No relaxation
18.	Arsenic	0.05	No relaxation
19.	Cyanide	0.05	No relaxation
20.	Lead	0.1	No relaxation
21.	Zinc	5.0	May be extended up to 10.0
22.	Anionic detergents (MBAS)	0.2	May be relaxed up to 1
23.	Chromium as Cr^{+6}	0.05	No relaxation
24.	Poly nuclear aromatic Hydrocarbons	--	--
25.	Mineral Oil	0.01	May be relaxed up to 0.03
26.	Residual free Chlorine	0.2	Applicable only when water is chlorinated
27.	Pesticides	Absent	--
28.	Radio active	--	--

Table 10: Indian Drinking Water Standard (Source: IS 10500)

Please note that all figures in the table are in ppm or mg/L

The World Health Organization has a wealth of information. WHO's Guidelines for Drinking water Quality was set up in Geneva in 1993. These standards are the international reference point for drinking water standards.

For India these standards are set by the Bureau of Indian Standards (BIS), the erstwhile Indian Standard Institute (ISI).

The American Water Standards set by the internationally famous Environmental Protection Agency- the EPA.

In formulation of the standard for drinking water BIS has taken into consideration the following publications:

- International Standards for Drinking Water issued by World Health Organization, 1984.
- Manual of Standards of Quality for Drinking Water Supplies. Indian Council of Medical Research 1971.
- Manual on Water Supply and Treatment (third revision) CPHEEO, Ministry of Urban Development, 1989.

The Central Water Commission has recently come up with a document to present the tolerance limits for inland surface waters for the various classes of water use. As per ISI-IS: 2296-1982, the tolerance limits of parameters are specified as per classified use of water depending on various uses of water. The following classifications have been adopted in India:

- Class A: Drinking water source without conventional treatment but after disinfection
- Class B: Outdoor bathing
- Class C: Drinking water source with conventional treatment followed by disinfection.
- Class D: Fish culture and wild life propagation
- Class E: Irrigation, industrial cooling or controlled waste disposal

Work, Functions and Full forms are as:

BIS: Bureau of Indian Standards has specified drinking water quality standards in India to provide safe drinking water to the people.

CPCB: Central Pollution Control Board is monitoring the water quality of aquatic resources across the country.

WHO: World Health Organization is a specialized agency of the United Nations that is concerned with international public health. It was established on 7 April 1948, and is headquartered in Geneva, Switzerland. WHO is a member of the United Nations

EPA: In the United States of America, the Safe Drinking Water Act (SDWA) directs the U.S. **Environmental Protection Agency** to establish national standards for public drinking-water supplies.

ICMR: Indian Council of Medical Research Manual of Standards of quality for drinking water supplies govern by this council.

Here we have some parameters covered by BIS, CPCB and WHO in table 11, which are as follows:

Table 11: Showing some parameters covered by BIS, CPCB and WHO

Parameters	BIS		CPCB		WHO
	Desirable	Permissible	Drinking water source (with disinfection)	Drinking water source (with conventional treatment and disinfection)	
pH	6.5 – 8.5	No relaxation	6.5 – 8.5	6.0 – 9.0	6.5 – 8.5
DO (mg L^{-1})	6.0	–	6.0	4.0	–
TDS (mg L^{-1})	500	2000	500	1500	1000
Alkalinity-M (mmol L^{-1})	200	600	–	–	–
CO_3 Hardness (mg L^{-1} $CaCO_3$)	–	–	200	–	500
Total Hardness (mg L^{-1} $CaCO_3$)	300	600	200	–	500
Cl^-(mg L^{-1})	250	1000	250	600	250
NO_3^- (mg L^{-1})	45	No relaxation	20	50	10
Cr (mg L^{-1})	0.05	No relaxation	0.05	0.05	0.05

Here we have some parameters covered by USEPA, WHO, ISI, ICMR and CPCB in table 12, which are as follows:

Table 12: Showing some parameters covered by USEPA, WHO, ISI, ICMR and CPCB.

Parameters	USEPA	WHO	ISI	ICMR	CPCB
pH (mg/l)	6.5-8.5	6.5-8.5	6.5-8.5	6.5-9.2	6.5-8.5
Turbidity NTU	-	-	10	25	10
Conductivity (mg/l)	-	-	-	-	2000
Alkalinity (mg/l)	-	-	-	-	600
Total hardness (mg/l)	-	500	300	600	600
Iron *mg/l)	-	0.1	0.3	1.0	1.0
Chlorides (mg/l)	250	200	250	1000	1000
Nitrate (mg/l)	-	-	45	100	100
Sulfate (mg/l)	-	-	150	400	400
Residual (mg/l) free Chlorine	-	-	0.2	-	-
Calcium (mg/l)	-	75	75	200	200
Magnesium (mg/l)	-	50	30	-	100
Copper (mg/l)	1.3	1.0	0.05	1.5	1.5
Fluoride (mg/l)	4.0	1.5	0.6-1.2	1.5	1.5
Mercury (mg/l)	0.002	0.001	0.001	0.001	No relaxation
Cadmium (mg/l)	0.005	0.005	0.01	0.01	No relaxation
Selenium (mg/l)	0.05	0.01	-	-	No relaxation
Arsenic (mg/l)	0.05	0.05	0.05	0.05	No relaxation
Lead (mg/l)	-	0.05	0.10	0.05	No relaxation
Zinc (mg/l)	-	5.0	5.0	0.10	15.0
Chromium (mg/l)	0.1	-	0.05	-	No relaxation
E. coli (MPN/100 ml)	-	-	-	-	No relaxation

5.6. Water Treatment Methods for Domestic Use

Most of the methods of water treatment methods discussed below are Point of Use (POU) devices. **POU methods treat water at the point where is used** frequently at the kitchen sink. Only the water that is actually used for drinking, cooking, beverage preparation, etc. is treated. Most people using public water do not need to employ Point of Entry treatment devices or the more expensive POU devices like distillation and reverse osmosis. The contaminants most people using public water would be liable to experience at harmful or unacceptable levels are:

- Residual disinfectants (chlorine and/or chloramine, for example) added to keep water safe during distribution.
- Disinfection by-products, like the trihalomethanes.
- Lead (as discussed elsewhere, many homes leach lead into the water from pipes and/or fixtures).
- Brief, accidental contamination by microbes (*E. coli*, *giardia*, *cryptosporidia*, etc.) or other contaminants

5.6.1 Whole House Water Treatment or Treatment at Point of Entry (POE)

It is also called whole house water treatment, where all water entering the home is treated and is indicated when the water has problems that affect all areas of the home. The most common example is a water softening ion exchange system that removes calcium and magnesium ions and some other ions from the water. Hard water, while quite healthy to drink, can cause scale build-up in pipes and on fixtures, interfere with the effectiveness of soap, and shorten the life of appliances, like dish washers and hot water heaters. Other POE water treatment systems are designed to remove iron and manganese, adjust pH levels, add chlorine or other disinfectant etc.

5.6.2 Water Treatment at Point of Use (POU)

Methods of drinking water treatment at point of use are as follows:

a) Boiling Treatment: Boiling drinking water with fuel is the oldest and most commonly practiced household water treatment method. According to WHO, water needs to be heated until the appearance of the first big bubbles to ensure that it is pathogen free. Boiling only kills pathogens and does not remove turbidity or chemical pollution from drinking water. So prior to boiling, water can be purified by settling or filtration method. It is oldest and most effective household drinking water treatment.

Boiling is one of the most effective water treatment methods to kill or deactivate all classes of waterborne pathogens, including bacterial spores and protozoan cysts that have shown resistance to chemical disinfection and viruses that are too small to be mechanically removed by microfiltration. Heating water to even 55 °C has been shown to kill or inactivate most pathogenic bacteria, viruses, helminths and protozoans.

This technique is easy, simple and widely accepted method of disinfection.

b) UV-Light Treatment: The bactericidal effect of concentrated ultraviolet (UV) light is used in many areas and for drinking water treatment, simple, commercially available UV tubes can be used to kill pathogenic microorganisms. Such UV tube water disinfection devices are an effective, low-cost and simple mean for a very rapid disinfection. They generally consist of a pipe, through which water slowly flows, and in which an UV light bulb is installed, which can be run on electric or solar power.

A typical UV system provides a flow path surrounding a UV lamp, structured to provide close proximity of the water flow along the length of the UV lamp all by preventing direct contact, see fig.02.

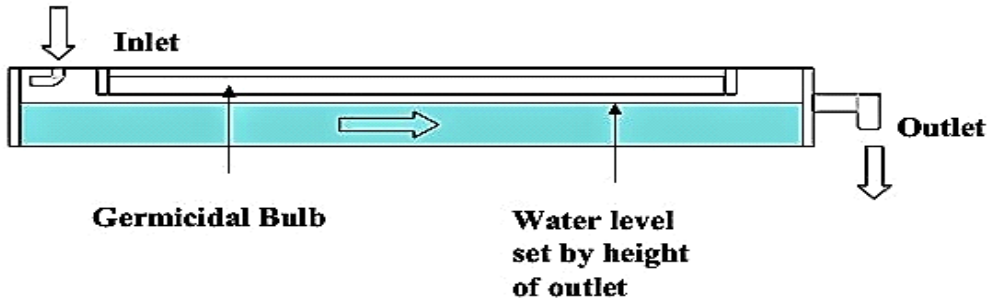


Figure 02: Concept of UV Tube Design.

Working of UV radiation disinfection: The UV tube is basically the same as a commercial fluorescent bulb, except that it lacks the phosphor coating and the glass exterior is replaced by fused quartz. This means the bulb emits mostly UV light. UV light is generally defined to be wavelength of electromagnetic radiation shorter than 400 nm and is further divided into UV-A (315-400nm), UV-B (280-315nm) and UV-C (200-280 nm).

The bulbs are suspended inside a larger tube in a covered channel. Water enters at one end and flows through the outlet at the other end. While the water flows through the tube, the UV light emitted from the bulb inactivates the microorganisms. The inactivation is directly related to continuous UV dose and depends on the intensity of the UV light and the duration of UV exposition.

UV tubes are effective in inactivating most pathogens, including bacteria, viruses, and cyst forming protozoa such as cryptosporidium. Yet, the effectiveness depends strongly on the UV dose.

c) Chlorination Treatment: The disinfection of drinking water by adding chlorine is called chlorination. Chlorine was used for the first time in 1850 when John Snow in London's water distribution system to combat cholera. Similarly, American cities like Chicago and New Jersey started to use chlorination around 1908, a step which brought a significant decrease in the number of deaths caused by cholera, typhoid, diarrhoea and hepatitis A. Today, chlorination is used to treat most of drinking water in the world since it is easy, inexpensive and reliable.

In addition to destroying harmful microorganisms, chlorination also reduces the amount of iron, manganese and hydrogen sulphide in water.

Chlorination is widely applied for the centralised disinfection of drinking water in municipal water supply systems.

Chlorine as a household level point of use treatment is available either a solution which is added at doses of one to several drops per litre of water to treat, or as tablets, which will dissolve in the treated water. Aside from these commercial products, water can also be treated at the community level by mixing chlorine in water tanks, wells and household vessels.

Working of chlorination: When chlorine is added to water, the chemical element dissolves and forms radicals. These kill pathogens such as bacteria and viruses by breaking the chemical bonds in their molecules or by attacking the cells of the microorganisms.

Chlorine disinfection of drinking water is limited for the protozoan pathogens and some viruses. Turbidity can protect microorganisms from disinfection. How to treat water with chlorine tablets is shown in fig. 03.

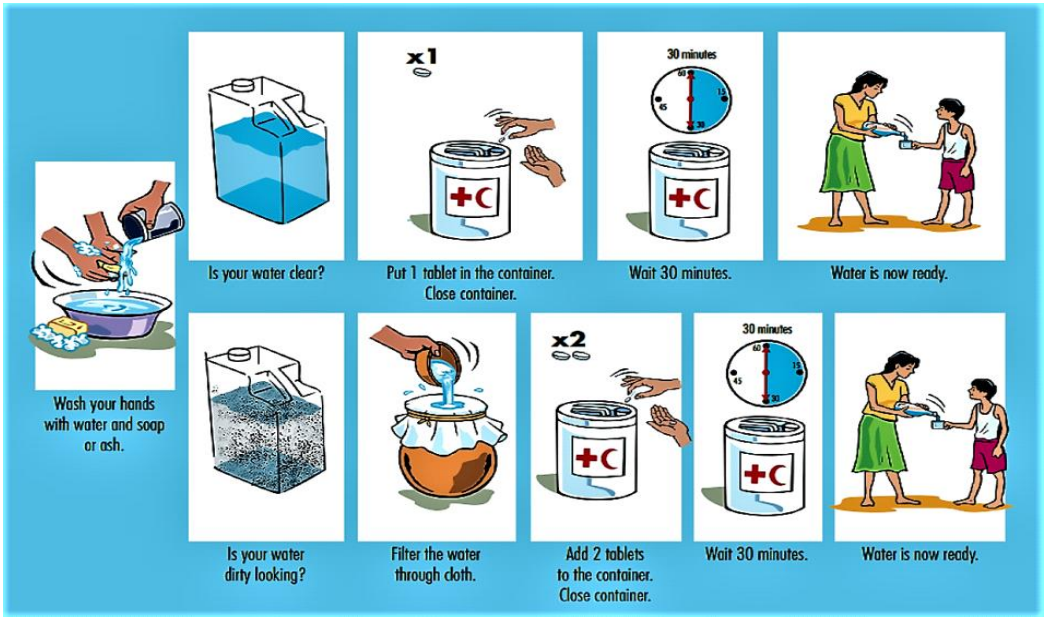


Figure 03: Showing How to treat water with chlorine tablets. Source: IFRC (2008)

d) Sedimentation: Sedimentation is a simple, low cost pre-treatment technology to reduce settable solids and some microbes from water under the influence of gravity prior to application of other purification methods. It also improves the visual qualities of the water and increases its acceptance by consumers. The longer the water is stored, the more the suspended solids and pathogens will settle to the bottom of the container. Adding chemical or natural coagulants to the water can quicken the sedimentation process. Aluminium sulphate, polyaluminium chloride and ferric sulphate are three common types of chemicals used for the coagulation. Some examples of natural coagulants are prickly pear cactus, Moringa seeds, broad beans and Fava beans. After sedimentation, the water should be filtered to further remove suspended materials and pathogens.

Coagulants enhance sedimentation because they neutralise the surface charge of suspended particles. Particles that cause turbidity (e.g. silt, clay) are generally negatively charged, making it difficult for them to clump together because of electrostatic repulsion. But chemical coagulant particles are positively charged, and they chemically attracted to negative turbidity particles, neutralising the latter's negative charge and accumulate to form larger particles (flocs), which settle faster, see fig 04.

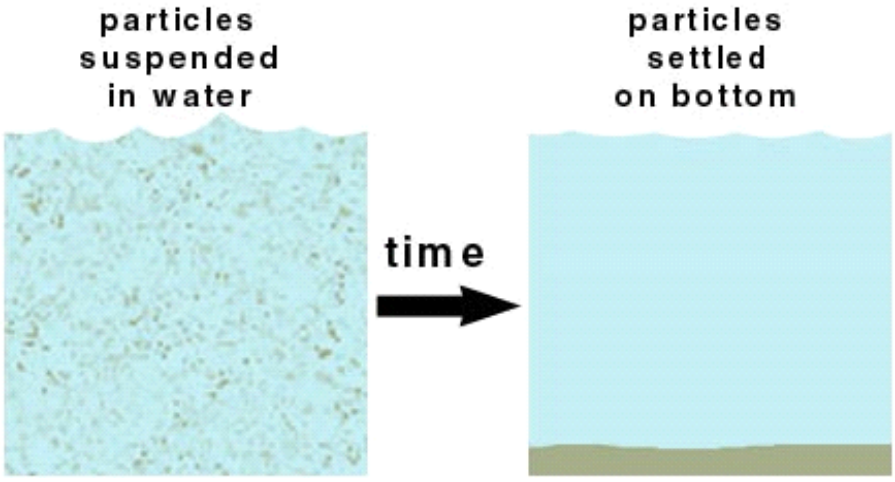


Figure 04: Showing Sedimentation treatment at home

e) Ozonation: Ozone is a gas composed of three oxygen atoms (O3), which is one of the most powerful oxidants. Ozonation is a type of **advanced oxidation process**, involving the production of very reactive oxygen species able to attack a wide range of organic compounds and all microorganisms.

Working of ozonation: Ozone has powerful oxidising effect on chemicals and microorganisms caused by the generation of reactive oxygen species during ozone transformation to oxygen. Ozone directly attacks the surface of microorganisms and destroys their cell walls. The cells thus loose their cytoplasm and can no longer reactivate themselves. Ozone can induce an oxidative degradation of many organics and leaves more biodegradable compounds. Besides, ozone can oxidise metallic ions such as Fe(II), Mn(II) or As(III) producing insoluble solid oxides that can be easily separated from water by filtration or sedimentation. So various types of contaminations remove by ozonation, see fig. 05.

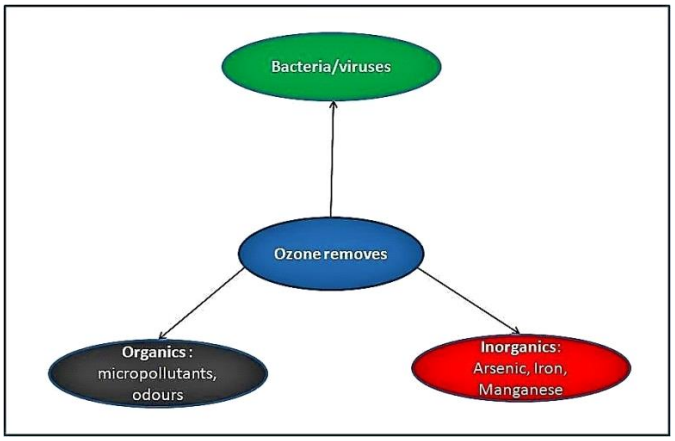


Figure 05: Showing Type of contaminants removed by ozonation.

Ozone rapidly reacts with bacteria, viruses and protozoa over a wide pH range. It has stronger germicidal properties than chlorination, in it no chemicals are added to water. It also efficient for organics degradation and inorganics removal. It removes colour, taste and odour.

f) Membrane Filtration: Membranes are becoming increasingly popular for production of potable drinking water from ground, surface and seawater sources, as well as for the advanced treatment of wastewater.

Membranes are thin and porous sheets of material able to separate contaminants from water when a driving force is applied. Once considered a viable technology only for desalination, membrane processes are increasingly employed in both drinking water and wastewater treatment for removal of bacteria and other microorganisms, particulate material, micropollutants, and natural organic material, which can impart colour, tastes, and odours to the water and react with disinfectants to form disinfection by-products (DBP).

Membrane processes are of high performance and have compact units, in them less space needed than conventional treatment schemes. These are simply operative. Membranes available can be used to separate many kinds of contaminants from drinking water. In it disinfection can be performed without chemicals.

g) Advanced Oxidation Processes: These Processes are efficient methods to remove organic contamination not degradable by means of biological processes. AOPs are a set of processes involving the production of very reactive oxygen species able to destroy a wide range of organic compounds.

Advanced Oxidation Processes (AOPs) refer to a set of oxidative water treatments that can be used to treat toxic effluents at industrial level, hospitals and wastewater treatment plants. AOPs are successful to transform toxic organic compounds (e.g. drugs, pesticides, endocrine disruptors etc.) into biodegradable substances. AOPs in general are cheap to install but involve high operating costs due to the input of chemicals and energy required. The combination of several AOPs is an efficient way to increase pollutant removal and reduce costs, see fig. 06.

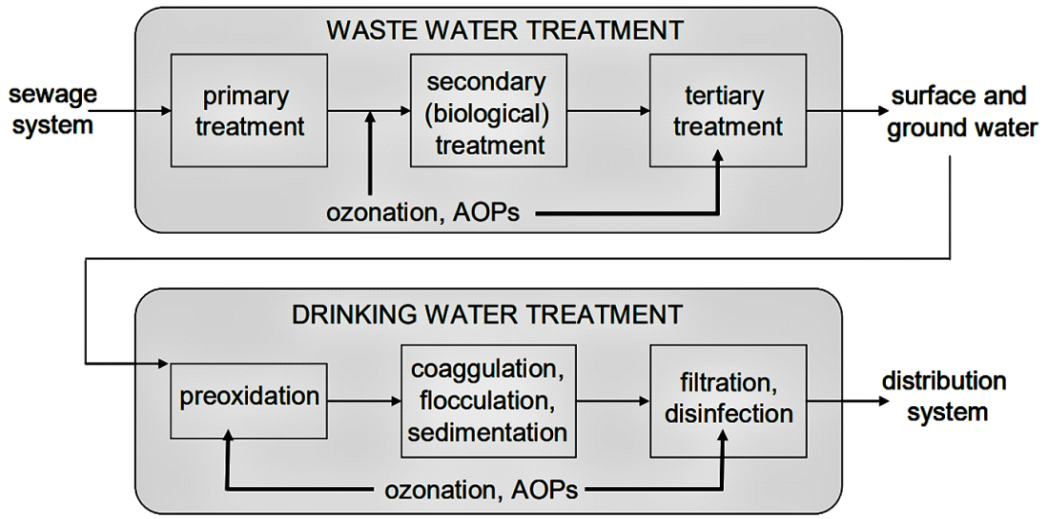


Figure 06: Showing Possible applications of ozonation and AOPs in wastewater and drinking water treatment.

Advanced Oxidation Processes (AOPs) are cheap to install.

h) Advanced Filters: The most common technologies applied in these filters, either alone or in combination, are sedimentation, activated carbon, membranes, ceramics, and Ultra Violet radiation. They are applicable in households, schools, small communities or hospitals.

Tap water is very often not safe enough to drink, due to microbial and chemical contaminants, which endanger human health.

The aim for all of these technologies is the same: to make sure that people all around world have access to save drinking water. These filters are of following types as:

I) Reverse osmosis filter, II) Tap filter, III) Tulip water filter.

I) Reverse osmosis filter: Reverse osmosis filter method is very efficient method to purify water from even very small molecules. It is possible to combine reversed osmosis filters with UV, infrared technology, or ozonation for disinfection. Filters have 4 to 6 filter stages in different combinations:

Stage 1 (5 Micron Sediment Filter): Removes dirt, rust and sand particles.

Stage 2 (Granular Activated Carbon Filter or Carbon Block Filter): Takes out 99% of the chlorine and organic chemicals and provides enhanced reduction of taste, odour, and colour.

Stage 3 (1Micron Sediment Filter): Provides effective filtration to protect the membrane.

Stage 4 (Reverse Osmosis (RO) Membrane): A thin film composite (TFC) high quality membrane processes 80 gallons (300 litres) per day. It removes the following hard water contaminants that may be present in the water: lead, cooper, barium, chromium, mercury, sodium, cadmium, fluoride, nitrite, nitrate, and selenium, see fig. 07.

Additional Filter Stages: Stage 5 (Post Carbon Filter): Removes objectionable tastes and odours to enhance the quality of drinking water.

Stage 6a (Deionisation (DI) Filter): Produces 99.99% pure water by simply attaching this convenient deionisation filter with RO unit. A convenient post RO filtration DI unit (RO/DI unit) provides crucial supplemental filtration to remove most impurities for pure polished product water. Excellent in areas with hard water.

Stage 6c (Mineral Filter): This filter improves the qualities of clean water by adding minerals which are necessary for proper human development and health, such as calcium, magnesium, sodium, potassium and others readily found in many natural mineral waters.

Stage 6d (Ultraviolet Water Steriliser): Ultraviolet light (UV), a natural part of sunlight is widely accepted as a reliable, efficient & environmentally friendly solution for water

disinfection. The UV lamp destroys 99.99% of bacteria and viruses. Fig.07

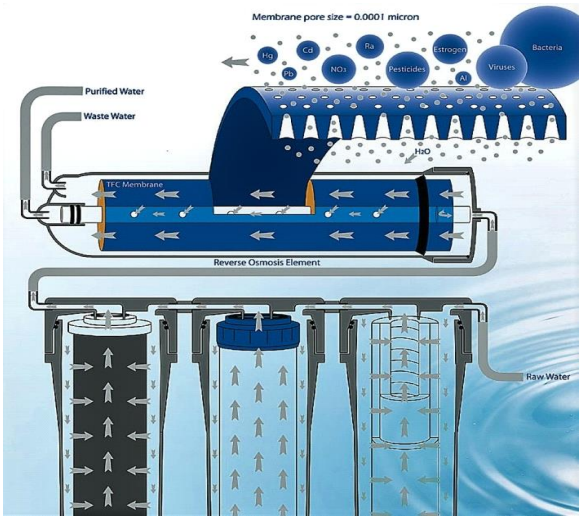


Figure 07: Showing A possible design for a 4 stage reversed osmosis filter

II) Tap filter: Tap filters are filters that can be fitted directly onto a faucet. They are easy to handle and quite effective since they remove pathogens, contaminants (e.g. chlorine, lead, asbestos), sediments and bad odours. The user can turn the filter on and off by switching a small handle.

III) Tulip water filter: The Tulip water filter is a candle-type water filter, which uses gravity siphon pressure to force water through a high-quality ceramic filter element. The innovative usage of the siphon results in a high flow rate of 4-5 litres per hour. The filter is impregnated with silver in order to increase the bacterial removal efficiency of the filter and to reduce the recontamination risk of stored filtered water. The filter must be cleaned by backwashing because sediments can lead to clogging. Ceramic filters remove pathogens effectively,see fig.08

Figure 08: Showing 1. Upper container with contaminated water 2. Plastic jar with filter element and washable pre-filter 3. Plastic connector 4. Rubber bulb to start the filtering process and to clean the element; 5. Opening and closing tap 6. Lower container with lid and tap for storage of drinking water.

i) Ion Exchange: In 1850, Thomas and Way performed some of the first scientific research that indicated the existence of an ion exchange process. Hard water can be softened using an ion exchange softening process.

A typical example of ion exchange is a process called “water softening” aiming to reduce calcium and magnesium content. Nevertheless, ion exchange is also efficient in removing toxic metals from water.

Ion exchange processes can also remove various charged atoms or molecules (ions) such as nitrates, fluoride, sulphates, perchlorate, iron and manganese ions as well as toxic metals (radium, uranium, chromium, etc.) from water.

Working of Ion exchange process: The main component of ion exchange equipment is a microporous exchange resin, which is supersaturated with a loosely held solution. As water passes through this resin bed, ions attach to the resin beads releasing the loosely held solution into the water.

After a time, the beds become saturated and the exchange resin must be regenerated or recharged. To regenerate, the ion exchange resin is flushed with a salt brine solution. The sodium ions in the salt brine solution are exchanged with the ions, which are flushed out with wastewater, see fig. 09

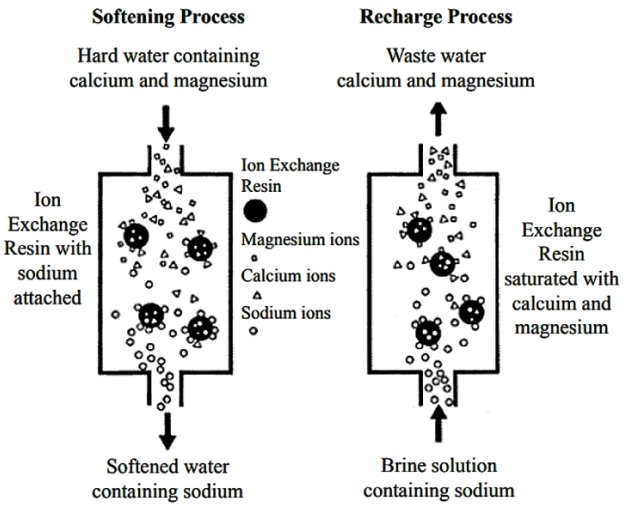


Figure 09: Showing the water softening and recharge process.

It is one of the most appropriate technologies to removes dissolved inorganic ions effectively. It has the possibility to regenerate resin.

j) Reverse Osmosis (RO): In this method purified water is collected from the "clean" side of the membrane, and water containing the concentrated contaminants is flushed down the drain from the "contaminated" side. The average RO system is a unit consisting of a sediment/chlorine pre filter, the reverse-osmosis membrane, a water storage tank, and an activated-carbon post filter.

Reverse osmosis significantly reduces salt, most other inorganic material present in the water, and some organic compounds. Microscopic parasites (including viruses) are usually removed by properly functioning RO units, but any defect in the membrane would allow these organisms to flow undetected into the "filtered" water. see fig. 10

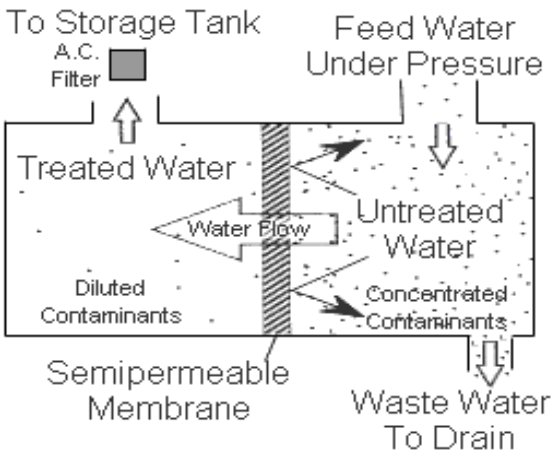


Figure10: Showing reverse osmosis process

5.7 Limitations

There are some limitations for drinking water treatment methods, which are as follows:

a) Limitation of Boiling: Some of the limitation for the same are as: this technique is costly due to the fuel consumptions. Use of traditional fuel (firewood, kerosene/gas) contributes to deforestation and indoor air pollution which is dangerous for environmental issues. Risk of injuries is there especially when children are around. This technique does not remove turbidity, chemicals, taste, smell, colour of drinking water and time consuming. Water needs to cool down before use unless for hot drinks.

b) Limitation of UV-tubes: Equipment cost is high and it requires regular power source for operation. In it lamp tube needs replacement every 6-12 months. Some investment for installation is required for this technique. UV lamp needs to be cleaned regularly and handled with care because of their mercury content. This technique is effective only for microbial pollution. No residual disinfection effect and risk of re-growth or recontamination.

c) Limitation of Chlorination: In this technique the users purchase chlorine on a continuous basis and may not affordable by very poor people. Availability of chlorine may be restricted in rural and remote areas. It requires clear water to be most effective. Chlorination of water with high organic matter leads to the risk of toxic disinfection by-products formation.

d) Limitation of Sedimentation: If only settling or plain sedimentation is practised, it removes only partially turbidity and some microorganisms. Maximum effectiveness requires careful control of coagulant dose and pH, and consideration of the quality of the water being treated, as well as mixing. The costs are variable depending on coagulant; some coagulants like polyelectrolyte are expensive to buy. Effectiveness of coagulants varies from one to another. In this technique, without using coagulants, a long sedimentation time is needed. It is not effective for removing dissolved chemicals from the water

e) Limitation of Ozonation: Equipment cost is high and requires large amounts of energy. In this technique qualified professionals required for design and system maintenance. Formation of potentially harmful disinfection by-products (DBPs) in the case of brome existence in water.

f) Limitation of Membrane Filtration: Membrane fouling occurs, production of polluted water (from backwashing) takes place. The membranes have to be replaced on a regular basis

g) Limitation of Advanced Oxidation Processes: Relatively high operation costs due to chemicals and/or energy input. In it the formation of oxidation intermediates are potentially toxic.

h) Limitation of Advanced Filters: Some of the introduced systems are very expensive (e.g. reverse osmosis filters), the spare parts are not easily available in case of failure. Correct instruction necessary and maintenance often difficult. This technique often requires electrical power.

i) Limitation of Ion Exchange: It does not remove effectively bacteria and having high operation costs over long-term. The process of regenerating the ion exchange beds dumps salt water into the environment (regeneration) which is harmful.

k) Limitation of Reverse Osmosis (RO): Most point of use RO units make only 12 - 24 gallons of treated water a day for drinking or cooking, which is ok for most homes since the treated water is stored in a tank for use. RO systems waste water. Two to four gallons of "waste" water are flushed down the drain for each gallon of filtered water produced. RO systems require maintenance. The pre and post filters and the reverse osmosis membranes must be changed according to the manufacturer's recommendation, and the storage tank must be cleaned periodically. Damaged membranes are not easily detected, so it is hard to tell if the system is functioning normally and safely.

Summary

- In this unit we have discussed about Drinking Water treatment and about the different forms of microbes found in drinking water.
- This unit is also able to describe the Indicator Organisms of drinking water.
- We have also understood about Total Coliform, Coliforms, Faecal Coliforms, Escherichia Coli, Faecal Streptococci, Enterococci or Intestinal Enterococci, Sulphite-reducing clostridia (SRC), Clostridium perfringens and Bacteriophages (phages).

- We have also focused on different bacteriological analytical techniques one of them was Multiple fermentation tube technique Or MPN Technique (MPN Index).
- Different Water quality standards for drinking water were also studied along with full forms of different organizations providing different parameters of drinking water for good health of humans.
- We have also discussed about several water treatment methods for domestic use of water viz. Boiling Treatment, UV-Light Treatment, Chlorination Sedimentation, Ozonation, Membrane Filtration, Advanced Oxidation Processes, Advanced Filters as: Reverse osmosis filter, Tap filter, Tulip water filter and Ion Exchange and Reverse Osmosis.
- We have also studied about the limitations of several water treatment methods for domestic use of water.

Terminal Questions

Q.1.) Fill in the blanks

..... contamination cannot be detected by sight, smell, or taste. The only way to know if a water supply contains bacteria is to have it tested by a qualified **.....**. The presence of faecal coliform in drinking water is a strong indication of recent **.....** or animal waste contamination. ..... (*E. coli*) bacteria is a subgroup of faecal coliform. *E.coli* outbreaks related to food **.....** have received media attention. These outbreaks are often caused by a specific strain of *E. coli* known as **.....** *E. coli* (STEC). When a drinking water sample is reported as "*E. coli*. present," it does not necessarily mean that this specific strain is present. However, it does indicate recent faecal contamination, which should be interpreted as an indication that there is a greater risk that **.....** are present. **.....** bacteria are non-coliform species of bacteria that use an organic substance for their development. Heterotrophic bacteria can be widespread throughout a water system. The presence of heterotrophic bacteria in **.....** is not an indication that the water presents a **.....**. Rather, no specific significance or health standards are associated with these **.....**, non-coliform bacteria.

Q.2.) What do you mean by microbes present in drinking water?

Q.3.) Describe indicator organisms.

Q.4.) Explain MPN Technique or MPN Index in detail.

Q.5.) What is meant by water quality standards for drinking water?

Q.6.) Explain Point Of Entry (POE).

Q.7.) Fill in the blanks

When ..... is added to ....., the chemical ..... dissolves and forms ..... These ..... pathogens such as ..... and ..... by breaking the ..... in their molecules or by attacking the cells of the ..... The different radicals and ions formed during ..... destroy many bacteria and viruses, but also oxidise some organic matter, dissolve colours and ..... chloramines, toxic products derived from ..... It takes about 30 minutes to do this work and make water ..... to .....

Q.8.) Explain chlorination of drinking water.

Q.9.) Explain Ozonation.

Q.10.) What do you mean by limitations of water treatment methods ?

Answers

Q.1.) Bacterial, laboratory, Escherichia coli, contamination, Shiga toxin-producing, pathogens, Heterotrophic, drinking water, health risk, non-pathogenic.

Q.2.) Pls . refer 5.2

Q.3.) Pls . refer 5.3

Q.4.) Pls . refer 5.4.1

Q.5.) Pls . refer 5.5

Q.6.) Pls . refer 5.6.1

Q.7.) Chlorine, water, element, radicals, kill, bacteria, viruses, chemical bonds, microorganisms, chlorination, destroy, ammonia, safe, drink.

Q.8.) Pls . refer 5.6.1.c

Q.9.) Pls . refer 5.6.1.e

Q.10.) Pls . refer 5.7

Unit 6: Detoxification of pollutants

Unit Structure

6.0 Learning Objectives

After studying this unit you would be able to:

- Understand the mechanism of detoxification
- Types of organic pollutants.
- explain the methods of degradation of highly toxic pollutants
- illustrate the microbial cell / enzyme technology and role of extracellular polymers

6.1 Introduction

We know that the limited availability of fresh water is a global crisis. The growing consumption of fresh water by anthropogenic activities has taken its toll on available water resources. Unfortunately, water bodies are still used as sinks for wastewater from domestic and industrial sources. However, in recent times, the need to replenish our water resources has been receiving increasing attention. This has led to the development of strategies to

return water to its source in the least toxic by detoxification of pollutants. The strategies and processes may be collectively termed as 'wastewater treatment'.

This unit attempts to explore and evaluate the types of pollutants and mechanism of detoxification. In this unit we also study the degradation of highly toxic pollutants along with degradation techniques

Types of microbial enzymes and technologies used for enzymatic treatment of wastewater will also be discussed. This unit also focused on the advantages of enzymatic treatment over other techniques and contribution of EPS to microbial cell aggregation.

6.2. Types of pollutants

Many different chemicals are regarded as pollutants, ranging from simple inorganic ions to complex organic molecules. The pollutants are all divided up into various types. Every class of pollutants has its own specific ways of entering the environment and its own specific dangers. All classes have major pollutants in it that are known to many people, because of the various health effects. The pollutants are of following types as:

6.2.1 Organic pollutants

Organic compounds are compounds that consist of long bonds, usually made up of carbon. Many organic compounds are basic fabrics of living organisms. Molecules built of carbon and of carbon and hydrogen are non-polar and have little to no water solubility. They have little to no electrical charge. The behaviour of organic compounds is dependent upon their molecular structure, size and shape and the presence of functional groups that are important determinants of toxicity. It is important to know the structure of organic compounds, in order to predict their fate in living organisms and the environment. The organic compounds that are dangerous to the environment are all man-made and have only existed during the last century.

Types of Organic pollutants

There are many different types of organic pollutants, examples are:
A) Hydrocarbons: These are carbon-hydrogen bonds. They can be divided up into two classes, the first being single-bonded alkanes, double bonded alkenes and triple bonded alkynes (gasses or liquids) and the second being aromatic hydrocarbons, which contain ring

structures (liquids or solids). Aromatic hydrocarbons such as PAH's are much more reactive than any of the first class kinds of hydrocarbons.

B) PCB's: These are stable and unreactive fluids that are used as hydraulic fluids, coolant/ insulation fluids in transformers and plasticizers in paints. There are many different PCB's. None of them are water-soluble. In many countries PCB's are restricted.
C) Insecticides: As DDT's are very dangerous because they accumulate in fat tissues of lower animals and then enter the food chain. They have been restricted for decades.
D) Detergents: These can be both polar and non-polar.

6.2.2 Types of inprganic pollutants

A) Inorganic fertilizers: Some inorganic pollutants are not particularly toxic, but are still a danger to the environment because they are used so extensively. These include fertilizers, such as nitrates and phosphates. Nitrates and phosphates cause algal blooms in surface water, which causes the oxygen level of the water to decline. This causes oxygen starvation because of the uptake of oxygen by microorganisms that break down algae. This is called **eutrophication**.

B) Metals: The first class we will refer to here is metals. Metals are good conductors of electricity and generally enter chemical reactions as positive ions, known as cations. Metals are natural substances that have consisted through weathering of ore bodies, where they were deposited during volcanic action. They can be relocated into situations where they can cause serious environmental damage. Examples of metals are: lead, zinc, manganese, calcium and potassium.

They can be found in surface waters in their stable ionic forms. Unnatural metals can be very dangerous, because they often come from man-made nuclear reactions and can be strongly radioactive. Metals can react to dangerous products with other ions. They are often involved in electron transfer reactions involving oxygen. This can lead to the formation of toxic oxyradicals. Metals can form metalloids and then bond to organic compounds to form lipophilic substances that are often highly toxic and can be stored in the fat-supply of animals and humans. Metals can also bond to cellular macromolecules in the human body. They have a density greater than 5 and are therefore called heavy metals, cannot be broken down

into less harmful components, as they are non-biodegradable. Organisms need metals, as they are essential for their health and are usually essential components of enzymes.

C) Radioactive isotopes: The half-lives and the ways of decay of radioactive isotopes determine how dangerous they are to humans. Humans create all radioactive isotopes in the nuclear industry. There are still debates going on about whether the benefits of nuclear power exceed the dangers of radioactive radiation. When an atom of a radioactive substance decays, it can produce four kinds of particles: alpha, beta, gamma and neutrons.

Alpha particles can only travel a short distance through air and human tissues, but they can be very damaging if they collide with cells because of their large mass. They are positively charged.

Beta particles are more penetrating, but they do much less damage than alpha particles. They are negatively charged.

Gamma rays are highly penetrating. Their damage is similar to that of beta rays. Neutrons are liberated through radiation and react with other elements through collision. They are the basis for nuclear fission in a reactor. The radioactivity of a substance is measured in becquerel. Different kinds of radiation can do different kinds of damage, because the energy is imparted into tissues in different ways. This is expressed in sieverts. An amount of alpha radiation can do twenty times the damage of the same amount of beta radiation. Radioactive matter has to be held in storage for different periods of time, in order to erase the danger. How long it has to be stored depends upon the half-life of the isotopes; the time taken for half of the atoms of a radioactive isotope to decay.

6.3 Mechanism of detoxification

In microbial degradation the wastes can usually be broken down or even consumed as food by some organisms. When degraded, the waste may be made less toxic and its harmful effects reduced. Remineralisation is the complete breakdown of an organic chemical (for example, into its basic components such as carbon dioxide, nitrogen, phosphorus, and water), such as may occur by microbial digestion. Remineralisation completely destroys toxicity inherent in such waste. Most remineralization is conducted by microbes (primarily

bacteria and fungi). The ability of microbes to metabolize and remineralize different wastes is highly variable.

Remineralisation depends on the type and number of microbes in the total community that are capable of degrading a particular waste. The number of waste degrading microbes in turn depends on prior exposure to the waste (or similar types of waste) through chronic or single-event exposures. Degradation of the wastes may increase after exposure as a result of an increase in number of waste-degrading microbes or the induction of appropriate enzyme systems. The rates at which microbes can degrade wastes are influenced by temperature and thus may change seasonally. The presence of oxygen often exerts a strong influence on degradation rates, and wastes may persist for very long periods of time in sediments that have little or no oxygen, even though microbes in oxygen-rich environments may readily degrade the same waste.

Marine and aquatic sediments and water-saturated soils in wetlands are often low in oxygen. Hence, the water availability in a specific location, acting through changes in the amount of saturated sediments and soils or even through changes in soil moisture, may have significant effects on contaminant degradation rates. Some wastes break down in anaerobic conditions. Specifically, the removal of chlorine atoms from PCB molecules, a necessary first step in their remineralisation, seems to occur (though slowly) in anaerobic conditions. High concentrations of waste contaminants may also be toxic to the bacteria and fungi that could otherwise degrade the contaminant where it presents in lower concentrations. This restricts the breakdown of a contaminant to the less contaminated areas of a site, slowing the overall degradation, see table 01.

Table 01: Showing Processes Involved in Waste Processing and Detoxification

Processes	**Factors Affecting Processes**
Contaminant movements in water bodies	
Mixing or dilution	volume of receiving waters, stratification
Advection and dispersion	velocity of water, turbulence regime
Water residence time	water input rate, tidal height, salinity distribution

Particle and sediment interactions and scavenging to sediments	solubility of contaminant (Kow), particle deposition rate
Sediment-water exchange	diffusion rate, bioturbation rate, water level fluctuations
Sequestering of chemicals (acid volatile sulfides in sediments)	oxidation state of sediments, availability of sulfides
Scavenging to (accumulation at) sea–air interface	solubility of contaminant
Volatilization	vapor pressure of contaminant, wind speed and water surface roughness
Aerosol formation	breaking waves
Precipitation from solution	solubility limits
Biological transports	uptake by organisms, settling of organic materials, food chain transfers

Chemicals that can be used as food by microbes tend to break down more quickly than poor food-quality chemicals. Some organic chemicals are especially slow to break down in the environment. For many of these, their resistance to microbial degradation results from the presence of many attached chlorine or bromine atoms. While there are many naturally occurring organic compounds incorporating chlorine or bromine which degrades, albeit slowly, naturally many of those synthesized by the chemical industry are quite resistant to microbial degradation.

The chlorine carbon bonds in these synthetic chemicals are not naturally abundant, and organisms do not have enzymes effective at degrading these chemicals. One group of such resistant chemicals with known toxic effects are the persistent organic pollutants, some of which have been banned from further production by international treaties. The time required for the concentration of POPs in a contaminated area to decrease measurably is typically measured in decades. The decrease is partly due to slow degradation of the PCBs but also to their dilution and spreading.

Where large amounts of waste are present, such as in an oil spill, the degradation rates of the wastes may be limited by factors needed for microbial growth such as nitrogen and phosphorus. If bacteria cannot increase in number, the degradation rates of compounds will remain slow. The physical nature of spills may also inhibit microbial breakdown. If the spilled waste remains in large clumps, or patches, the microbes cannot reach or degrade the interior of the clump, and only the surface of the clump is subject to microbial decay, greatly slowing the digestion of the waste.

For many organic wastes, and particularly large (high molecular weight) compounds, it is important to distinguish between an initial alteration of the chemicals in the waste (a relatively small change in the form of the waste) and complete remineralization. A small alteration of a parent compound may result in persistent and/or toxic daughter products that may be of as much concern as the parent waste chemical. With such a complex array of factors that can influence biodegradation, it is not usually a simple matter to predict the persistence of a degradable waste in an environment. However, experience with wastes has been gained and models have been developed that allow such predictions to be made with good confidence.

6.3.1. Degradation of highly toxic pollutants

What is toxic pollution?

Toxic pollution is contaminated water, soil, and air that is harmful or poisonous. It includes industrial wastes like toxic heavy metals from mining or chemicals from factories, and also sewage and particulates from power plants. The term “toxic” is used to differentiate it from pollution that comes from increased levels of carbon dioxide, which causes climate change but does not have direct health impacts.

Toxic pollutants can poison drinking water, the fish in rivers and ponds, food grown on contaminated farmland, as well as playgrounds, homes, and the very air we breathe.

In fact, toxic pollution is the largest cause of death in the world. Yet it is one of the most underreported and underfunded global problems.

How does toxic pollution affect public health?

Pollution comes in many forms, and it affects people differently, sometimes in ways that may not be immediately noticeable. That is why some people call toxic pollution “the invisible killer."

Pollution can cause birth defects and irreversible developmental and neurological disabilities, and immune system damage. Pollution causes various cancers, heart and lung diseases, to name just a few. Comparatively, death by pollution is larger than any other major cause. Often whole communities are affected, and economic growth impaired as well through degradation of human and natural resources.

6.3.2. Degradation Techniques

We know that continuous increase of pollutants in water bodies has necessitated the need to develop cost-effective methods for their removal. Destroying the pollutants to benign chemicals and/or removing these pollutants from contaminated water is imperative for a green environment. There are various treatment processes that have been applied for pollutant removal from wastewater, such as electrochemical oxidation, biodegradation membrane process, coagulation, adsorption, precipitation, sonochemical degradation, micellar enhanced ultrafiltration, and AOP.

Though these methods are considered as efficient methods for pollutant removal, each method has its own benefits and drawbacks. In this section, we will explain some of the most common methods that are frequently used for pollutant removal and their basic principles.

a) Electrochemical Oxidation: It is an efficient and economic method, suitable when the wastewater contains non-biodegradable organic pollutants. This method poses several advantages since it does not require auxiliary chemicals, high pressures, or high temperatures. By its versatility and cost-effectiveness, electrochemical techniques have gained great attention for the removal of pollutants. The process of electrochemical oxidation mechanism is mainly based on the generation of the hydroxyl radicals at the electrode surface.

Two different types of mechanisms have been elaborated for electrochemical oxidation, such as **direct** and **indirect** oxidation methods.

In direct electrochemical oxidation, the degradation of organic compound occurs directly over the anode material, where the hydroxyl radical or the reactive oxygen species react

with the organic compound. The pollutants are first adsorbed at the surface of the anode and are then degraded by an anodic electron transfer reaction.

In the indirect electrochemical oxidation, the organics are treated in the bulk solution by oxidants, such as , Cl_2, hypochlorite, peroxodisulfate and ozone (O_3), which are electrochemically generated at the electrode surface. Even though high removal efficiencies are achieved by both the direct and indirect electrochemical oxidation processes, their effectiveness strongly depends on the treatment conditions including pH, current density, types and concentration of pollutants, supporting electrolyte, flow rate, electrode preparation method and nature of the electrode materials.

Several electrode materials that include Pt, PbO_2, Ti-SnO_2, Ti/Pt, Ti/Pt–Ir, Ti/PbO_2, Ti/PdO–Co_3O_4, Ti/–TiO_2, Ti coated oxides of Ru/Ir/Ta, IrO_2, Ti/RuO_2, SnO_2, PbO_2, and so forth, and boron doped diamond (BDD) have been listed as efficient electrodes for the degradation of organics by electrochemical oxidation. Apart from these anode materials, graphite anodes are also considered as efficient materials for anodic oxidation of several organic pollutants. Particularly, the high oxygen over potential, high electro catalytic activity, chemical stability, long lifetime, and cost-effectiveness has been credited for the high efficiency of these graphite electrodes, shown in fig. 01.

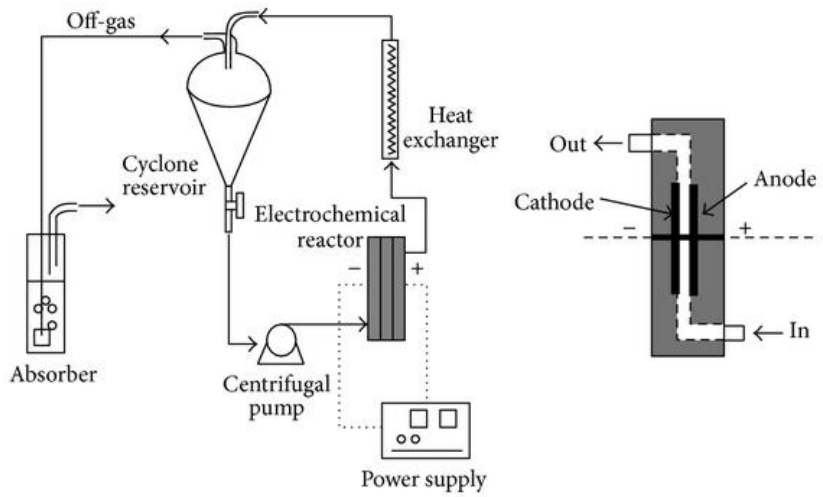


Figure 01: Showing the pilot plant arrangement and illustration of electrochemical oxidation

The electrochemical performance was examined by measuring COD and the concentrations of all phenolic compounds formed during the electrolysis process. However, the addition of chloride ion to the electrolyte solution caused a major change in the reaction kinetics. The difference in the reaction kinetics was explained by the oxidation mediated by the chloride ions, which was formed at low pH values. Though the concentration of chloride ions helped to rapidly increase the reaction rate, increment in the supporting electrolyte concentration also assisted to improve the reaction kinetics.

b) Biological Process: Biological degradation technology with high removal efficiency has been successfully applied in industries for effluents and waste gas treatments. Even though biological processes have been used for several applications that include heavy metal ion removal and indoor air purification, its application has not been intensely investigated for numerous reasons. Thus, biological process combined with other techniques such as chemical processes, photocatalysis and AOP.

Combined biological and chemical degradation methods were carried out to evaluate the effectiveness of mature municipal landfill leachate. The biological treatment was followed by chemical oxidation for further removal of COD. Higher removal efficiency was obtained due to the use of chemical treatment, a combined AOP and biological process was carried out to remove pesticides in aqueous solution. The experimental setup for their study is illustrated in Figure 02. The chemical oxidation process was carried out in the tank labelled as 1. The UV emitting device (labelled as 5) consists of a stainless-steel tube with a coaxial mercury vapour lamp. Ozone was produced from air by an ozone generator and was continuously fed into the oxidation tank. Finally, the pH of the system was adjusted to 7, and then the pollutant was fed to the biological treatment tank labelled as 9.

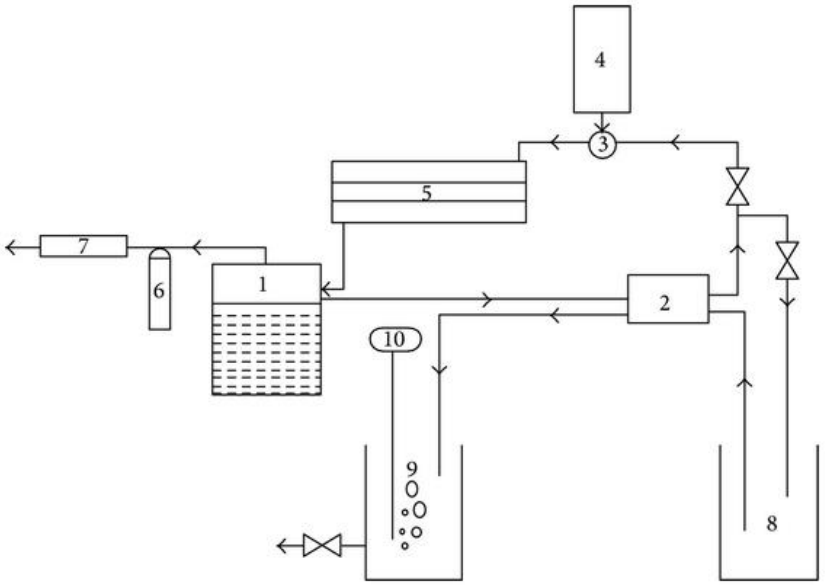


Figure 02: Showing experimental setup for combined chemical and biological degradation: chemical oxidation tank, peristaltic pump, diffuser valve, ozone generator, UV emitting device, excess KI trap, ozone destruction device, neutralizer, biological treatment tank, and air sparger.

c) O_3 and O_3/UV oxidation treatment: This O_3 and O_3/UV oxidation treatment was able to achieve 90 and 100% removal of the pesticide deltamethrin, in a period of 210 min. Utilization of ozone with UV irradiation was found to enhance the degradation of pesticides. It has been well documented elsewhere that the rate of pesticide removal mainly depends on both the chemical nature of the pesticides being treated and the treatment conditions. Under their reaction conditions, pentachlorophenol degraded faster compared with other phenolic compounds and 4-chlorophenol degraded the slowest.

A combined biological and chemical procedure was also used as an ecologically and economically favourable remediation technique for 2,4,6-trinitrotoluene (TNT) reduction in contaminated ground and surface water. It was observed that the anaerobic transformation process resulted in a faster reduction of TNT due to significant change in the redox potential of the solutions under both aerobic and anaerobic conditions. Biodegradation of organic pollutants by halophilic bacteria.

Wastewater from a pharmaceutical formulation facility was treated with a biological activated sludge system followed by ozonation. The by-products identified from the incomplete

oxidation of ozonation were likely to be more biodegradable than the parent compounds; thus, a postozonation biological treatment was endorsed for the efficient removal of these toxic pharmaceuticals from the wastewater.

d) Adsorption: This method is an effective and well-known process and has been widely explored as an alternate technique compared with the other waste removal methods due to the lower cost, flexibility and simplicity of design, and ease of operation. Moreover, adsorption does not result in production of any harmful substances. Discharge of several types of pollutants that include household wastes, phenolic wastes, dyes, pesticides, herbicides, and metal ions into the water body causes health issues not only for humans but also for aquatic life.

Dyes have been identified as a major contaminant in wastewater. Many industries, such as textile, leather, paper, plastics, food, and cosmetics, use several dyes as coloring materials. Most of these dyes are very toxic, and when released into the environment, they can be transported over long distances in water sources resulting in their widespread dispersal. More than a million tonnes of dyes and coloring materials are produced annually, and the drinking water quality is greatly affected by the unsafe release of these dyes into the water body. In addition, the presence of even very small amounts (<1 ppm) of dyes, in particular the synthetic dyes, in water is undesirable. Therefore, the presence of dyes in wastewater is a major concern for toxicological and esthetical reasons.

Adsorption is a method that is capable of removing nondegradable waste pollutants. There are several adsorbents that include clay minerals, activated carbon, coal, wood, fly ash, and biomaterials that have been listed for the removal of industrial wastes. However, due to the lack of effective adsorbate-adsorbent interactions, some of the above-mentioned materials are found to be non-effective for the adsorption of pollutants. Consequently, oxide materials, which are considered as an important class of adsorbents, have been explored for adsorption.

e) Advanced Oxidation Processes (AOP): In this process oxidation takes place, which typically uses ambient conditions (room temperature and atmospheric pressure). Several AOP techniques such as ozonation, H_2O_2 photolysis process and heterogeneous

photocatalysis have been explored for the elimination of pollutants, particularly from water sources. These AOP techniques destroy the pollutants by chemical oxidation or reduction.

In particular, AOP relies on the production of hydroxyl radicals, which are short-lived, extremely highly reactive species and attack most organic molecules with rate constants of . Moreover, the versatility of AOP is enhanced by different possible ways for the production of hydroxyl radicals

f) Ozonation: We know that Ozone is unstable in water and the molecular ozone can react as a dipole, electrophile, or nucleophile due to the two different resonance structures. In addition, depending on the pH, temperature, and concentration of organic and inorganic compounds in water, the half-life of ozone varies from a few seconds up to a few minutes. Ozone is a powerful oxidant and it can oxidize a large number of organic and inorganic materials. Ozone reacts either directly or indirectly with aqueous compounds. In the direct reaction, the molecular ozone directly reacts with the compounds, whereas the radicals resulting from the decomposition of ozone.

In this process the direct reactions are very slow and solute selective, whereas the indirect radical reactions are fast and nonselective. Additionally, the direct reactions are dominant in acidic solutions, while the indirect reactions occur mostly at basic pH values. Ozonation of phenol gave catechol, hydroquinone, benzoquinone, maleic acid, and oxalic acid as the ring cleavage intermediate products and it was noticed that some of the intermediates such as catechol, hydroquinone, and benzoquinone can be destroyed completely using ozone to CO_2 and H_2O. However, destruction requires long ozonation time and high dosage of ozone. The hydroxyl radicals are the active species in the decomposition of organics.

g) UV/H2O2 Treatment: This treatment in AOP also involves the formation of radicals generated by the photolysis of H_2O_2 in the presence of UV irradiation and is very effective for the degradation of most of the organic pollutants. Owing to the higher molar absorption coefficient of the peroxide anion, the photolysis rate has been found to be pH dependent and increases at high pH values. Owing to the commercial availability, thermal stability, infinite solubility in water, storage, and ease of formation of hydroxyl radical, the use of hydrogen peroxide as an oxidant has received significant attention for water purification.

However, the rate of formation of hydroxyl radicals influences the oxidation of organic contaminants and this has been noted as the main disadvantage of the UV/H_2O_2 treatment process. According to this study, an acidic medium is more effective for the degradation of DMSO.

There are a large number of organic pollutants, such as phenols and phenolic compounds, benzene and substituted benzene, salicylic acid, proline, pyridine, and dyes, that have been degraded or mineralized using UV/H_2O_2 treatment. However, this treatment method is not the focus of this study. In addition to the UV/H_2O_2 treatment for oxidation of pollutants, another process involves the use of O_3/UV. Even though H_2O_2increases the generation of radicals in the ozone process. The H_2O_2/UV method removed 99% of total organic carbon (TOC) from the effluent.

6.4. Microbial cell/enzyme technology

Enzymes are biocatalysts produced by living cells to cause specific biochemical reactions.These are highly specific in their action on substrates and often many different enzymes are required to bring about sequence of metabolic reactions performed by living cells.

Each strain of a microorganism produces a large number of enzymes which can be hydrolysing, oxidizing or reducing and metabolic in nature. Microbial enzymes are known to play a crucial role as metabolic catalysts, resulting in their use in various industrial applications. The end use market for industrial enzymes is extremely widespread with numerous industrial commercial applications. Microbes have served and continue to serve as one of the largest and useful sources of many enzymes.

Many industrial processes have several disadvantages like low catalytic efficiency, lack of enantiomeric specificity for chiral synthesis, requirement of high temperature, low pH and high pressure. Also, the use of organic solvents leads to organic waste and pollutants. Enzymes are more useful for these applications as they work under mild reaction conditions (e.g., temperature, pH, atmospheric conditions).

Enzymes can be selected genetically and chemically-modified in order to improve their key properties: stability, substrate specificity and specific activity. In the case of ocean and river

quality, pollution is primarily caused by the discharge of inadequately treated industrial and municipal wastewater.

On initial discharge, these wastewaters can contain high levels of inorganic pollutants which can be easily biodegraded, but whose impact load on the ecosystems, either in Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), or Chemical Oxygen Demand (COD), may be in the tens of thousands mg/L. To combat this increasing burden on our aquatic environment, increasingly strict regulation on pollution discharge is being implemented by various governmental bodies, with focus primarily on waste reduction. Compliance to environmental legislations should not necessarily lead to the creation of additional costs, but can instead provide a secondary source of income. One possible source of increased revenue available to industries is through taking advantage of the incentives awarded by the Clean Development Mechanism (CDM) under the Kyoto Protocol 1997.

The characteristics of wastewater can differ considerably both within and among industries. In the treatment of wastewater, biological treatment appears to be a promising technology to attain revenue from Certified Emission Reduction (CER) credits, more commonly known as carbon credits, as methane gas is generated from anaerobic digestion and can be utilized as renewable energy. With appropriate analysis and environmental control, almost all wastewaters containing biodegradable constituents with a BOD/COD ratio of 0.5 or greater can be treated easily by biological means.

In comparison to other methods of wastewater treatment, it also has the advantages of lower treatment costs with no secondary pollution. Both aerobic and anaerobic processes can be used; the former involves microorganisms (aerobes) which utilise free or dissolved oxygen and convert organic wastes to biomass and CO2 while in the latter complex organic wastes are degraded into methane, CO2 and H2O through three basic steps (hydrolysis, acidogenesis including acetogenesis and methanogenesis) in the absence of oxygen. Aerobic biological processes are commonly used in the treatment of organic wastewaters for achieving high degree of treatment efficiency, while in anaerobic treatment, considerable progress has been achieved in anaerobic biotechnology for waste treatment based on the concept of resource recovery and utilization while still achieving the objective of pollution control.

Most of the waste treatment processes can be categorized as either physico-chemical or biological processes. Enzymatic treatment falls between these two conventional categories since it involves chemical processes based on the action of biological catalysts. The potential advantages of enzymatic treatment as compared with conventional treatment include: application to bio refractory compounds; operation at high and low contaminant concentrations; operation over a wide range of pH, temperature and salinity; absence of shock loading effects; absence of delays associated with the acclimatization of biomass; reduction in sludge volume and the ease and simplicity of controlling the process.

6.4.1. Types of microbial enzymes

a) Microbial oxidoreductases: The detoxification of toxic organic compounds by various bacteria and fungi and higher plants through oxidative coupling is mediated with oxidoreductases.

b) Microbial oxygenases: Oxygenases belong to the oxidoreductase group of enzymes and participate in oxidation of reduced substrates by transferring oxygen from molecular oxygen (O2) utilizing FAD/NADH/NADPH as a co-substrate. These enzymes have an important role in the metabolism of organic compounds as they increase their reactivity or solubility in water or bring about cleavage of the aromatic rings. Oxygenases also mediate dehalogenation reactions of halogenated methanes, ethanes, and ethylenes in association with multifunctional enzymes.

c) Monooxygenases: These enzymes catalyze oxidative reactions of substrates from alkanes to complex molecules as steroids and fatty acids and require only molecular oxygen for their activity. These enzymes require only molecular oxygen for their activities and utilize the substrate as reducing agent. The desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation, and biodegradation of various aromatic and aliphatic compounds are catalyzed by monooxygenases.

d) Microbial dioxygenases: These dioxygenases catalyze enantio specifically the oxygenation of wide range of substrates. Aromatic compounds are primarily oxidized by dioxygenases, reflecting the applications of dioxygenases in environmental remediation. The catechol dioxygenases are found in the soil bacteria and involved in the transformation of aromatic precursors into aliphatic products.

e) Microbial laccases: Laccases (p-diphenol: dioxygen oxidoreductase) constitute a family of multicopper oxidases produced by certain plants, fungi, insects, and bacteria, that catalyze the oxidation of a wide range of reduced phenolic and aromatic substrates with concomitant reduction of molecular oxygen to water. Many microorganisms produce intra and extracellular laccases capable of catalyzing the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins, and aryl diamines as well as some inorganic ions.

f) Microbial peroxidises: These enzymes catalyze the oxidation of lignin and other phenolic compounds at the expense of hydrogen peroxide (H2O2) in the presence of a mediator. Due to their high potential to degrade toxic substances in nature, lignin peroxidase (LiP) and manganese-dependant peroxidase (MnP) have been studied the most.

g) Microbial lipases: These enzymes can catalyze various reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis. Along with its diagnostic usage in bioremediation, lipase has many potential applications in food, chemical, detergent manufacturing, cosmetic, and paper making industries, but its production cost has restricted its industrial use.

h) Microbial cellulases: During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol. Cellulases cause removal of cellulose microfibrils, which are formed during washing and the use of cotton-based cloths. In paper and pulp industry, cellulases are used for the removal of ink during recycling of paper.

i) Microbial proteases: Proteases belong to group of enzymes that hydrolyze peptide bonds in aqueous environment and synthesize them in nonaqueous environment. Proteases have wide range of applications in food, leather, detergent, and pharmaceutical industry (Table 02).

Industries and pollutants Aromatic compounds, including phenols and aromatic amines, comprise one of the major classes of pollutants and are stringently regulated in many countries. They are found in the wastewaters of a wide variety of industries including coal conversion, petroleum refining, resins and plastics, wood preservation, metal coating, dyes and other chemicals, textiles, mining and dressing, and pulp and paper. The Kraft process

which is widely used in wood pulping leaves 5-8% (w/w) of residual modified lignin in the pulp.

This residual is responsible for the characteristic brown colour of the pulp and is commercially removed by the use of bleaching agents such as chlorine and chlorine oxides. Bleaching operations produce dark brown coloured effluents which contain toxic and mutagenic chlorinated products that constitute an environmental hazard.

There have been a number of studies including the use of peroxidases and laccases for the treatment of bleaching effluents. Pesticides, which include herbicides, insecticides and fungicides, are widely used throughout the world today for crop protection and it is expected that this use will continue to grow. The potential adverse effects that the pesticide industry can have on the environment arise from the disposal of wastes formed during production and formulation of pesticides, detoxification of pesticide containers and spray tanks, and the pollution of surface and groundwater by pesticide runoff. It is estimated that 3 million tons of cyanide are used yearly throughout the world in different industrial processes including the production of chemical intermediates, synthetic fibers, rubber and pharmaceuticals, as well as in ore leaching, coal processing and metal plating.

Enzymes could be used to decrease food wastes via enzymatic processing to yield higher-value by-products and to aid in the clean-up of food waste streams. For the past decade, there has been an increasing interest in the enzymatic hydrolysis of cellulose. This interest stems from the advantages that such a process would offer, namely, the conversion of lignocellulosic and cellulosic wastes to a useful energy source through the production of sugars, ethanol, biogas or other energetic end products.

Heavy metals such as arsenic, copper, cadmium, lead and chromium among others, are dangerous contaminants found in a number of industrial and mining waste streams as well as in solid wastes, municipal sewage sludges and landfill leachate. Surfactants or surface active agents are organic substances that have rather large polar molecules and are basic ingredients of detergents. Surfactants may cause significant pollution problems when high concentrations from shampoo formulation factories, for instance, enter municipal sewerage systems and generate undesirable conditions such as foaming. Wastewaters from dairies

and slaughterhouses are often rich in biodegradable organic molecules and nutrients and contain high levels of fats and proteins that have a low biodegradability coefficient.

Some dyestuffs are highly structured polymers and are very difficult to decompose. Many of the dyes are carcinogenic, mutagenic and detrimental to the environment. Leather processing industries are also a huge source of waste which include wastes from untanned hides/skins (trimmings, fleshing wastes), wastes from tanned leather (shaving wastes, buffing dust), wastes from dyed and finished leather (trimmings from leather) toluene and benzene, shown in table 02.

Table 02: Showing microbial enzymes and applications

Enzymes	**Applications**
Alkyl sulfatase	Surfactant degradation
Amylase: a-amylase, Glucoamylase	Starch hydrolysis and production of glucose
Cellulolytic enzymes: Cellulase, Cellobio-hydrolase, Cellobiase, Exo-1,4-b-D-glucosidase	Hydrolysis of cellulosic sludges from pulp and paper to produce sugars and alcohol, hydrolysis of cellulose in municipal solid waste to sugars and other energy sources
Chitinase	Bioconversion of shellfish waste to N-acetyl glucosamine
Chloro-peroxidase	Oxidation of phenolic compounds
Cyanidase	Cyanide decomposition
Cyanide hydratase	Cyanide hydrolysis
L-Galactono-lactone oxidase	Conversion of galactose from whey hydrolysis to L-ascorbic acid
Laccase	Removal of phenols, decolourization of Kraft bleaching effluents, binding of phenols and aromatic amines with humus
Lactases	Dairy waste processing and production of value-added products

Lignin peroxidise	Removal of phenols and aromatic compounds, decolourization of Kraft bleaching effluents
Lipase	Improved sludge dewatering
Lysozyme	Improved sludge dewatering
Mn-peroxidase	Oxidation of monoaromatic phenols and aromatic dyes
Parathion hydrolase	Hydrolyzation of organophosphate pesticides
Pectin Lyase	Pectin degradation
Peroxidase	Removal of phenols and aromatic amines, decolourization of Kraft bleaching effluents, sludge dewatering
Phosphatase	Removal of heavy metals
Proteases	Solubilisation of fish and meat remains Improving sludge dewatering
Tyrosinase	Removal of phenols

6.4.2. Technologies used for enzymatic treatment of wastewater

The conformation of enzymes mainly decides their functionality. The conformation might change under harsh physical and chemical conditions of temperature, pH and ionic strength thus altering the function of enzymes. Such drastic conditions are often encountered in effluent streams.

a) Immobilization method: It reduces the loss of enzymes increasing their reusability and also minimizes the chances of loss of enzyme activity under harsh conditions. Use of immobilized enzymes in effluent treatment, as compared to free enzymes, results in multiple advantages like increased stability, reusability, ease of handling, reduction in running cost.

b) Horseradish peroxidise: Covalently immobilized onto magnetic beads retained a high activity and stability and performed higher phenol conversions.

c) Fungal laccase: Introduction of cells or tissues producing an enzyme into the effluent directly is one of the simplest method of enzyme administration to target effluent. This

method is employed when suitably adapted strains of microorganisms are used to co metabolize target pollutants.

In cases where the effluent to be treated contains pollutants which cannot support growth, cell free or isolated enzymes are preferred for use over intact microorganism. Nanotechnology is another area which is gaining importance in wastewater treatment. The use of nanoparticles in Reactive Remediation Technology is of great interest to wastewater treatment, since it involves the complete degradation of contaminants to harmless products such as carbon dioxide and water.

The remediation of contaminated wastewater can be achieved by using a combination of enzyme technology and nanotechnology known as the SEN, i.e., Single Enzyme Nanoparticle. A SEN may be described as an enzyme covered by a protective few nanometers thick cage. Cell free crude extracts or purified forms of enzymes like peroxidases, polyphenol oxidases, dehalogenases, hydrolases can be used for SEN synthesis. A variety of recalcitrant compounds such as phenols, polyaromatic dyes, pesticides can be degraded by these enzymes.

Similarly, recalcitrant pollutants in wastewater can also be treated by using nanotubes carrying oxidative enzymes as laccases and peroxidases. Membrane bioreactors constitute an interesting possibility to be applied in wastewater treatment. The combination of membrane technology with enzymatic reactors for wastewater treatment has led to the development of three generic systems: Immobilised enzyme membrane reactor (IEMR), Extractive membrane bioreactor (EMB) and Direct contact membrane reactor (DCMR).

6.4.3. Advantages of enzymatic treatment over other techniques

The continuously expanding application of enzymes is creating a growing demand for biocatalysts that exhibit improved or new properties. By specifically acting on certain recalcitrant pollutants, enzymes can remove them by precipitation or transformation to other products. Biological (enzymatic) processes have an added advantage over traditional chemical/physical methods as they are regarded as clean and green.

The various physicochemical treatments like chemical precipitation, coagulation, flocculation, floatation, membrane filtration offer advantages like ease of operation and control, flexibility to change in temperature and are rapid but their benefits, however, are

outweighed by a number of drawbacks such as their high operational costs due to the chemicals used, high energy consumption and handling costs for sludge disposal . Stringent government policies regarding permitted levels of pollutants, high costs of specialized chemical treatments for pollutant removal and the fact that some of these treatments create additional solid waste has led to the development of many effective, yet simple biological methods.

Enzymes are highly specific and extremely efficient catalysts. They can selectively degrade a target pollutant without affecting the other components in the effluent. More importantly, they can operate under mild reaction conditions, especially temperature and pH. In this respect, enzymes outperform the regular catalysts (transition elements like Cu, Ni etc.). From the environmental perspective, enzymes are more acceptable due to their biodegradability. In the case of reactions wherein the target pollutant is oxidized, the enzyme receives one or more electrons from the substrate and donates these electrons to an electron acceptor.

Hence, at the end of the reaction the enzyme is regenerated and is available for the next catalytic cycle. The biological origin of enzymes reduces their adverse impact on the environment thereby making enzymatic wastewater treatment an ecologically sustainable technique.

6.5. Role of extracellular polymers

Extracellular polymeric substances (EPS), as a mixture of polymers secreted from microorganisms, and produced from cellular lysis and hydrolysis of macromolecules, are the fundamental materials for the formation and stabilization of the structures of microbial aggregates. Thus, EPS play a very important role in the determination of physiochemical properties of microbial aggregates, such as conformation, adsorption, flocculation, settling, dewatering, and degradation properties, in various biological wastewater treatment processes.

6.5.1. What are EPS?

The majority of microorganisms exist in aggregated forms, such as biofilms, flocs, and granules, and extracellular polymeric substances (EPS) are the main fundamental materials

to keep cells together to form microbial aggregates. EPS were defined by Geesey (1982) **as "extracellular polymeric substances of biological origin that participate in the formation of microbial aggregates",** which has been observed and confirmed to be present in pure cultures, activated sludge, biofilms, and granules, using various electron microscopy techniques. Another definition is as**"all polymers outside the cell wall not directly anchored in the outer membrane/murein-protein-layer" should be considered as EPS**.

The EPS is a mixture of polymers mainly secreted from microorganisms, and produced by cellular lysis and hydrolysis of macromolecules, in addition to organic compounds absorbing from water phase. The forms of the EPS have generally been classified as two categories, including "bound EPS (sheaths, capsular polymers, condensed gels, loosely bound polymers, and attached organic materials) and soluble EPS (soluble macromolecules, colloids, and slimes)". Bound EPS can be subdivided into tightly 10 bound EPS (LB-EPS), which are closely adhered to the cell surface and stay in a certain shape in the inner layers of EPS matrix, and loosely bound EPS (LB-EPS), which are the outer layers of EPS that are loosely bound to the cells without certain edge.

Soluble EPS, which is identical to soluble microbial products (SMP) in literature, are soluble cellular components that released and dissolved into surrounding liquor. Figure 03, showing the definition of EPS structures. Generally, the way to separate bound EPS and soluble EPS is by centrifugation, where polymers remained in the supernatant are defined as soluble EPS and pellets formed by polymers are considered as bound EPS.

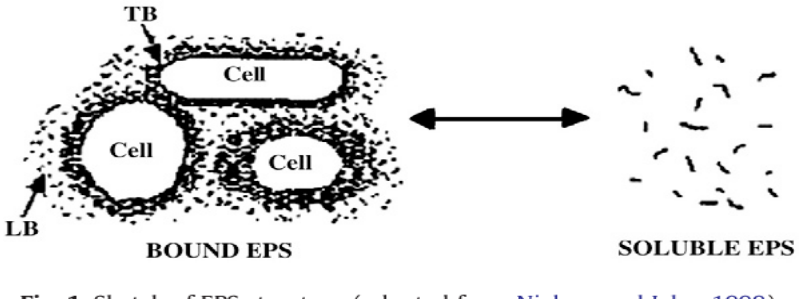


Figure 03: Showing EPS structure

EPS are a key factor that some strains used to bind Na^+ allowing their survival in high NaCl concentrations. Therefore, it is essential to understand the roles of EPS through

characterizing EPS in hypersaline wastewater treatment plant. In this work, comparison of the EPS composition and aggregation properties in hypersaline and municipal wastewater treatment plant was investigated to demonstrate the effect of salinity and dissolved oxygen on EPS characteristics.

Salinity stress has a negative effect on the biological treatment process. The high Na^+ concentration in the wastewater could exert the salinity pressure on the microorganism so that inhibited many kinds of enzymes activity, and decreased the microbial cells activity. The extracellular polysaccharide generated amount reduced to a half when the Na^+ 10 g/L increased to 20 g/L. However, the amount of total EPS increased with the NaCl concentration changed from 0 to 10.0 g/L in a biofilm reactor, in which EPS mixture (e.g., the extracellular polysaccharide) secretion was considered to be a protective response to the bacteria in the salinity pressure, as well as release of soluble microbial products and EPS also dramatically enhanced of the biomass to salinity stress in membrane bioreactor (MBR).

The total EPS content of salt tolerant bacterium, *Rhodopseudomonas acidophila*, increased with the NaCl concentration, also suggesting a protective response of the bacterium to the salinity. In terms of dissolved oxygen influence, It has been found that higher airflow rates increased the amount of carbohydrate in the bound EPS but the protein level was almost constant. More bound EPS provided more opportunities for cells to become/remain embedded in EPS, so that free cells or small aggregates were less. A higher microbial production of extracellular polymeric substances at a higher (4 mg/L) DO in high-loaded membrane bioreactors (HL-MBR) that gave a bigger mean floc size, a lower supernatant turbidity, better settleability and better membrane filterability than the HL-MBR that was operated at a DO of 1 mg/L.

The major functional groups of EPS were characterized by using Fourier transform-infrared spectroscopy (FT-IR) and three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectroscopy. In addition, the aggregation sedimentation performance of sludge flocs was investigated before and after EPS extraction as well.

In biological wastewater treatment systems, the majority of the microorganisms exist in the form of microbial aggregates, such as biofilms, flocs, and granules. In microbial aggregates,

extracellular polymeric substances (EPS), a mixture of biopolymers, provide the fundamental construction materials to form a three-dimension matrix to embed microorganisms and keep cells together, which determines the shape and mechanical stability of biological aggregates.

EPS act as the transfer medium that responsible for the import of nutrients and export of metabolic products between cells and the environment. EPS can also absorb exogenous organic compounds from the environment and digest the macromolecules into low molecular nutrients that can be easily utilized by cells. The functional groups (e.g. carboxyl, phosphoric, sulfhydryl, hydroxyl and amine groups) presence in EPS affect the surface charge of microbial aggregates and thus have significant influences on the adhesion, adsorption, hydrophilicity/hydrophobicity characteristics of microbial aggregates. Therefore, EPS play a critical role in the determination of physicochemical properties of biofilms or sludge flocs, such as conformation, adsorption, flocculation, settling, dewatering and degradation properties, in biological wastewater treatment systems.

The characteristics (e.g., adsorption abilities, biodegradability and hydrophilicity/hydrophobicity) and the contents of the main components (e.g., carbohydrates, proteins, humic substances and nucleic acids) in EPS are found to crucially affect the properties of microbial aggregates, such as mass transfer, surface characteristics, adsorption ability, stability, the formation of microbial aggregates etc. However, as EPS are very complex, the knowledge regarding EPS is far from complete and much work is still required to fully understand their precise roles in the biological treatment process.

In biological wastewater treatment, bacteria tend to aggregate by forming flocs, biofilms and even granules. A large part of the floc structure in activated sludge is composed of extracellular polymers. The extracellular polymeric substances (EPS) are mainly responsible for the structural and functional integrity of biofilms/flocs and they are considered to be important for the physico-chemical properties of activated sludge. This review considers the composition of EPS and proportion between the constituents.

The precise role of extracellular polymeric substances (EPS) in relation to the formation and physicochemical properties of microbial floc in wastewater treatment systems is not well known. Studies were undertaken to provide more comprehensive descriptions of EPS and

properties of microbial floc. Acidic polysaccharides and DNA were relatively labile components of the EPS when biomass was stored at 4°C or at –20°C, and significant losses of these components were observed within 24 hours.

The composition and properties of activated sludge were found to vary between different full-scale treatment systems reflecting the importance of wastewater composition and operation conditions on microbial communities and the response to environmental conditions. The COD:N:P ratio was found to influence hydrophobicity, surface charge and the EPS composition of microbial flocs in well-controlled bench-scale sequencing batch reactors. Phosphorus depleted and P-limited conditions resulted in a decrease in surface charge but increases in acidic polysaccharides which corresponded to a strong carboxyl stretch at 1740 cm^{-1} when the biomass was analysed by FTIR-spectroscopy. Electron dense particles, identified by energy-dispersive spectroscopy as containing iron, phosphorus and sulfur, were observed in the fibrils of the floc matrix by transmission electron microscopy.

6.5.2. Contribution of EPS to microbial cell aggregation

Many investigations had indicated that EPS has an important influence on the adhesion and aggregation of microbial cells. Especially, the TB-EPS had the greatest contribution on the aggregation and settlement and the aggregation process was described by pseudo first-order dynamic equation. In saline surroundings, microbes secreted more EPS and aggregated tightly to protect themselves both in MBR and A/O processes, and the sludge was quite compact figure 04 showed the aggregation capability of microbial cells suspension before and after EPS extraction from M-WWTP and H-WWTP (a. anaerobic tank in M-WWTP; b. anoxic tank in M-WWTP; c. oxic tank in M-WWTP; d. secondary sedimentation tank in M-WWTP; e. anaerobic tank in H-WWTP; f. oxic tank in H-WWTP; g. secondary sedimentation tank in H-WWTP).

It showed that the aggregation rates of each sample within 1 h were very high, whereas it almost could arrive at equilibrium state after 2 h. Besides, the aggregation rates of microbial cells suspension from the secondary sedimentation tank in M-WWTP, before and after EPS extraction, were much greater than that of other samples, which reached the equilibrium state less than 1 h. At the equilibrium state, the aggregation efficiency of each corresponding sample before EPS extraction was higher than after extraction, indicating that EPS was beneficial to the aggregation of microbial cells. See fig. 04

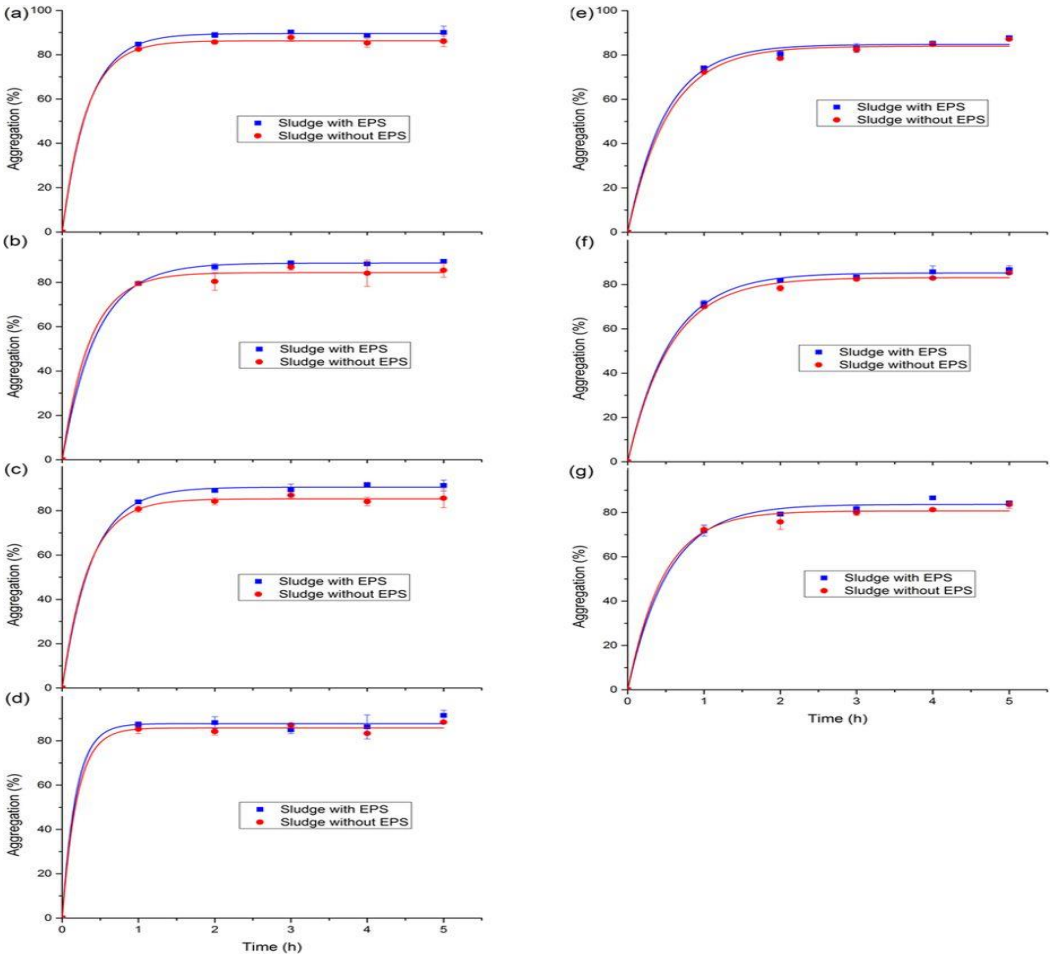


Figure 04: Figure showing composition and aggregation of extracelluar polymeric substance (EPS) in hyperhaline and municipal wastewater treatment plants.

6.5.3 Roles of EPS in microbial aggregates

After the observation and discovery of the presence of EPS-containing structures, the major functions of the EPS in microbial aggregates has been investigated and confirmed by several

studies (Table 03). In general, the most important role of EPS is supposed to be as the fundamental construction components for EPS matrix formation. A number of studies suggested that polysaccharides and lectin-like proteins play very important roles in the formation of three-dimensional networks of the EPS matrix by directly forming polysaccharide chains and protein-polysaccharide cross-links or indirectly via multivalent cation bridges.

Therefore, EPS have a significant effect on the physicochemical properties of microbial aggregates, such as structure, adsorption, flocculation, settling, dewatering and biodegradation properties. Thus, a deep study of the characteristics of EPS is of great importance to improve the understanding of the function and mechanism of EPS produced in bioreactors, which can promote the optimization of the design and operating parameters of biological wastewater treatment systems, observe table 03.

Table 03: Showing different roles of EPS

Roles of EPR	**Description**
Adhesion ability	The mediation of adherence to the cell surface to enhance the deposition and accumulation of bacteria on surfaces
Aggregation of microorganisms and formation of microbial aggregates	Structural elements of microbial aggregates; formation of a three-dimensional matrix to keep bacterial cells together to form biofilms and flocs; Bridging between cells and inorganic ions; determination of the shape of EPS structures and enhance the stability of microbial aggregates.
Mass transfer	The transfer medium responsible for the import of nutrients from substrates to cells and the export of metabolic products from cells to the environment; maintaining normal growth and metabolism of cells.
Sorption ability	Sorption and accumulation of organic compounds and inorganic ions trapped from the environment.
Enzymatic activity	Digestion of exogenous macromolecules to provide nutrients with low molecular weights that can be easily

	utilized by the cells; Degradation of Structural EPS in microbial aggregates to release cells.
Protective barrier	Protection of microorganisms against environmental stress and harmful substances, such as osmotic shocks, extreme temperature changes, and toxic substances.
Nutrition source	Serving as carbon or energy sources during nutrient shortage period.
Surface charge	The functional groups (e.g. carboxyl, phosphoric, sulfhydryl, phenolic, hydroxyl and amine groups) in EPS affect the surface charge of microbial aggregates and thus have significant influences on the adsorption, adhesion ability, hydrophilicity/hydrophobicity characteristic of microbial aggregates.
Retention of	Water Prevention of desiccation of cells during water-deficient environment; The macromolecules in EPS increase the water-holding capacity and affect the dewatering ability of sludge.

Role of EPS in biological wastewater treatment processes: Role of EPS in sludge flocculation, settling, and dewatering In general, EPS have been considered as an important factor in sludge flocculation. It has been reported that the ability of sludge flocculation was significantly affected by the interactions between EPS and cells. The mechanism of bio flocculation regarding EPS can be explained by the bridging between EPS and multivalent cations. Negatively charged functional groups in EPS tend to bridge multivalent cations, e.g. Ca2+ and Mg2+, and other particles with opposite surface charges to form flocculants.

The effectiveness of the bio flocculation caused by the bridging mechanism is affected by the molecular weight of EPS, the concentration of multivalent cations, the surface charge of the particles, and the degree of mixing. The sludge flocculation ability could be enhanced by directly adding multivalent cations in sludge. The removal of extracellular proteins resulted in the deflocculation of sludge and the releasing of polysaccharides, which indicated that proteins and polysaccharides were linked within the EPS matrix and played an important

role in bioflocculation. The flocculation ability of sludge had a positive correlation with the fraction of proteins and polysaccharides, and a negative relationship with the amounts of humic substances and total EPS contents.

The large number of EPS were reported to have a negative influence on the settling ability of sludge by several studies because negative charges of functional groups in EPS could increase the repulsive forces between cells and cause the poor performance of sludge settling. The LB-EPS had a great negative effect on the sludge settling as the loose structure of LB-EPS allowed more water to be retained within the floc matrix. Settle ability of sludge could also be affected by different EPS components. It has been reported that proteins and DNA had negative effects on the compressibility of sludge, however, the carbohydrate content did not show any significant correlation with sludge settling. The presence of macromolecules in EPS could increase the water-holding capacity of sludge and prevent the desiccation of cells during the water-deficient environment, which consequently decreases the dewatering ability of sludge.

The lower number of EPS had a positive effect on sludge flocculation as the cells attached more tightly within the EPS matrix, however, once the EPS content increased to exceed a certain amount, the ability of water retention of EPS significantly increased and led to a poor sludge dewatering ability. The different compositions of EPS also have different effects on sludge dewatering. Role of EPS in membrane fouling Membrane bioreactors (MBR) and anaerobic membrane bioreactors (AnMBR) have been widely used for wastewater treatment with various advantages, such as high quality effluent, decreased footprint, and reduced excess sludge production.

Membrane fouling has become of a great research interest in recent years as the occurrence of membrane fouling results in the increase of energy demands and operating costs. EPS have been reported to have an impact on fouling formation, and soluble EPS/SMP have been identified as the key biological factor that is responsible for membrane fouling by several studies. SMP can easily attach to the membrane to form a gel layer on the membrane surface and block 18 pores of the membrane, which provide a hydraulic resistance to the permeate flow, and thus result in the poor filterability of sludge.

The contributions of various SMP components to fouling are also different. Polysaccharides in SMP had more contribution to fouling than proteins. Table 03 summarized the main operating parameters.

Role of EPS in the absorption of metals and organic matters: The EPS in microbial aggregates have a number of functional groups, such as carboxyl, phosphoric, sulfhydryl, hydroxyl and amine groups, which have a high binding capacity to complex with heavy metals and organic substances and affect the transmission of these pollutants in wastewater. Extracellular carbohydrates, proteins, and nucleic acids all have binding capacities to form the complexes with heavy metals, and the mechanism of adsorption obeys the Langmuir adsorption equations. In addition, it has been suggested that SMP had a more significant adsorptive capability for heavy metals than bound EPS.

During the hydrolysis and digestion of sludge, the SMP content from the destruction of sludge was released into the liquid with a simultaneous increase of concentrations of metals, such as Ca2+ and Mg2+, which indicated the binding between soluble EPS and divalent cations played an important role in the formation of the structure of microbial aggregates.

The negatively charged EPS could also adsorb organic pollutants with positive charges, such as benzene, herbicide and dye from wastewater, through electrostatic interaction. The adsorption properties of EPS (from a sequencing batch biofilm reactor) found that 69%, 71% and 65% of benzene, toluene, and m-xylene were adsorbed by EPS, respectively, which could be explained by the binding at apolar groups from proteins and the formation of hydrophobic areas in the highly acetylated polysaccharides. Soluble EPS and bound EPS from activated 21 sludge and had strong adsorption ability. Soluble EPS had stronger binding capacity than bound EPS as the high fractions of proteins in soluble EPS could form more stable complexes with dicamba. They also found that proteins had the stronger binding capacity for dicamba than humic substances.

Recently, extracellular polymeric substances (EPS, biopolymers produced by the microorganisms) have been recognized as a potential flocculent for their applications in various water wastewater and sludge treatment processes. One of the most important aspects of chemical composition and structural details of different moieties of EPS in terms of carbohydrates, proteins, extracellular DNA, lipid and surfactants and humic

substances are described. These chemical characteristics of EPS in relation to formation and properties of microbial aggregates as well as degradation of EPS in the matrix (biomass, flocs etc) are analyzed. Different aspects of EPS production process such as bacterial strain maintenance, inoculum and factors affecting EPS production were presented. The important factors affecting EPS production include growth phase, carbon and nitrogen sources and their ratio, role of other nutrients (phosphorus, micronutrients/trace elements, and vitamins), impact of pH, temperature, metals, aerobic versus anaerobic conditions and pure and mixed culture. The production of EPS in high concentration with high productivity is essential due to economic reasons. Therefore, the knowledge about all the aspects of EPS production (listed above) is highly essential to formulate a logical and scientific basis for the research and industrial activities.

One of the very important issues in the production/application/biodegradation of EPS is how the EPS is extracted from the matrix or a culture broth. Moreover, EPS matrix available in different forms (crude, loosely bound, tightly bound, slime, capsular and purified) can be used as a bioflocculant material. Flocculability, dewaterability and biosorption ability are the very attractive engineering properties of the EPS matrix. Recent information on important aspects of these properties qualitatively as well as quantitatively has been described. The factors considered are cations, different forms of EPS, concentration of EPS, protein and carbohydrate content of EPS, molecular weight of EPS, pH of the suspension, temperature etc. Recently it has been demonstrated that there is an optimum EPS concentration for sludge flocculation/dewatering.

Summary

- In this unit we have discussed about detoxification of pollutants and about the types of pollutants.
- This unit is also able to describe the mechanism of detoxification.

- We have also understood about types of organic pollutants, degradation of highly toxic pollutants, degradation Techniques along with microbial cell/enzyme technology and types of microbial enzymes

- We have also focused on the technologies used for enzymatic treatment of wastewater and advantages of enzymatic treatment over other techniques
- We have also discussed about role of extracellular polymers with EPS
- We have also studied about contribution of EPS to microbial cell aggregation and roles of EPS in microbial aggregates

Terminal Questions

Q.1.) Fill in the blanks

In **……..** the wastes can usually be broken down or even consumed as food by some organisms. When degraded, the waste may be made less toxic and its harmful effects reduced. **……** is the complete breakdown of an organic chemical (for example, into its basic components such as carbon dioxide, nitrogen, phosphorus, and water), such as may occur by microbial digestion. Remineralisation completely destroys **……** inherent in such waste. Most remineralisation is conducted by microbes (primarily bacteria and fungi). The ability of microbes to metabolize and remineralize different wastes is highly variable.

Remineralization depends on the type and number of **……..** in the total community that are capable of degrading a particular waste. The number of waste degrading microbes in turn depends on prior exposure to the waste (or similar types of waste) through chronic or single-event exposures. **……** of the wastes may increase after exposure as a result of an increase in number of waste-degrading microbes or the induction of appropriate **……** systems. The rates at which microbes can degrade wastes are influenced by temperature and thus may change seasonally. The presence of oxygen often exerts a strong influence on degradation rates, and wastes may persist for very long periods of time in **……..** that have little or no oxygen, even though microbes in …….. environments may readily degrade the same waste.

Q.2.) What do you mean by types of Organic pollutants?

Q.3.) Describe types of microbial enzymes.

Q.4.) What are EPS?

Q.5.) Define roles of EPS in microbial aggregates.

Q.6.) What are the advantages of enzymatic treatment over other techniques?

Q.7.) Fill in the blanks:

…….. were defined by **…….** (1982) as "**……..** polymeric substances of biological origin that participate in the formation of microbial **……..**", which has been observed and confirmed to be present in pure **……..**, activated sludge, **……..**, and granules, using various electron **…….** techniques. Another definition is as"all **……** outside the cell wall not directly anchored in the **……** membrane/murein-protein-layer" should be considered as EPS. The EPS is a **……** of polymers mainly secreted from **……..**, and produced by cellular lysis and

hydrolysis of macromolecules, in addition to organic compounds absorbing from water phase.

Q.8) Explain degradation of highly toxic pollutants.

Q.9) Explain electrochemical Oxidation

Q.10) What do you mean by Advanced Oxidation Processes (AOP)?

Answers

Q.1.) Microbial degradation, Remineralisation, Microbes, Degradation, Enzymes, Sediments, Oxygen-rich.

Q.2.) Pls . refer 6.2.1

Q.3.) Pls . refer 6.4.1

Q.4.) Pls . refer 6.5.1

Q.5.) Pls . refer 6.5.3

Q.6.) Pls . refer 6.4.3.

Q.7.) EPS, Geesey, extracellular, aggregates, cultures, biofilms, microscopy, polymers, outer, mixture, microorganisms.

Q.8.) Pls . refer 6.3.1

Q.9.) Pls . refer 6.3.2.a

Q.10.) Pls . refer 6.3.2e

Unit 7 Waste water treatment

Unit Structure

7.0 Learning Objectives

After studying this unit you will be able to understand:

- What is sewage?
- What is the composition of wastewater
- About wastewater treatment
- Biodegradation of toxic pollutants
- Microbiology of biological treatment process

7.1 Introduction

As you know about the importance of water, it is one of the important natural resources to the existence of all living organisms, but this resource is increasingly being polluted as

human populations increase. Freshwater is only 2.5% at global level and very few amount of this freshwater is usable to drinking purpose. Among the various environmental issues of that India is facing fresh water scarcity.

Wastewater or sewage is the byproduct of domestic, industrial, commercial or agricultural activities. As you know domestic and industrial sewage is one of the major causes of water pollution. An estimated 38354 million litres per day (MLD) sewage is generated in major cities of India, but the sewage treatment capacity is only of 11786 MLD. With urbanization and domestic water supply, quantity of gray/wastewater is increasing in the same proportion. According to CPHEEO about 70-80% of total water supplied for domestic use gets generated as wastewater.

Sewage contains various impurities and when sewage enters into the aquatic bodies it causes eutrophication like condition. Sewage causes low dissolved oxygen, low pH, high turbidity, high biological oxygen demand, low transparency in aquatic ecosystems. Due to sewage discharge many important rivers of India such as Ganga, Yamuna, Gandak, Kaveri etc are depleting day by day. Industrial effluent or industrial sewage also contains various heavy metals such as cadmium, chromium, arsenic, mercury etc. These heavy metals are very harmful to human being and responsible for various diseases in human being. Heavy metals may reach to human being through biomagnification process. As you know biomagnification is increase the concentration of toxic substances in trophic level. Due to harmful nature of the wastewater it should be treated before release into aquatic ecosystem. In this unit you will learn about the wastewater treatment and role of microbes in biodegradation of toxic substances.

7.2 Wastewater and related terms

Wastewater can have a number of definitions. Some of the important definitions of wastewater are given here:

- Wastewater is domestic effluent consisting of black-water (excreta, urine and faecal sludge) and grey-water (kitchen and bathing wastewater);
- Water from commercial establishments and institutions, including hospitals;
- Industrial effluent, storm-water and other urban run-off;

- Agricultural, horticultural and aquaculture effluent, either dissolved or as suspended matter"

Wastewater is water in which physical, chemical or biological properties have been changed as a result of the introduction of certain impurities which render it unsafe for drinking purpose. As you know that human activities are water dependent and therefore discharge 'waste' into water. Some of the substances include body wastes (faeces and urine), detergents, food scraps, fat, laundry powder, fabric conditioners, toilet papers and micro-organisms which can cause serious diseases among communities and damage the environment. It is well known that much of water supplied ends up as wastewater which makes its treatment very important. ***Wastewater treatment is the process and technology that is used to remove most of the contaminants***.

There are following terms which are related to wastewater.

Stormwater Runoff: It is water from streets, open yard etc after a rainfall event which run through drains or sewers.

Industrial wastewater: It is liquid waste from industrial establishments such as factories, production units etc.

Domestic wastewater : It is also known as **municipal wastewater** is basically wastewater from houses, hotels and institutions. Further it can be categorized into grey-water and black-water.

Grey-water: It is also known as **sullage** is liquid waste from washrooms, laundries, kitchens which does not contain human or animal excreta.

Black-water: It is wastewater generated in toilets. Black-water may also contain some flush water besides urine and faeces. Urine and faeces together is sometimes referred to as **night soil**.

Sewage: It is the term used for black-water if it ends up in a sewerage system.

Septage: It is the term used for black-water if it ends up in a septic tank.

Effluent: It is the liquid stream which is discharged from a wastewater treatment plant or discharge from a unit process or operation.

Sludge: It is the semi-solid slurry from a wastewater treatment plant.

On-Site System: This is wastewater disposal method which takes place at the point of waste production like within individual houses without transportation. On- site methods include dry methods (pit latrines, composting toilets), water saving methods and methods with high water rise.

Off-Site System: In this system, wastewater is transported to a place either than the point of production. Off- site methods are bucket latrines, pour-flush toilets with vault and tanker removal and conventional sewerage system.

Unit Operation: This involves removal of contaminants by physical forces.

Unit Process: this involves biological and/or chemical removal of contaminants.

Wastewater Treatment Plant : It is a plant with a series of designed unit operations and processes that aims at reducing certain constituents of wastewater to acceptable levels.

7.3 Properties and Composition of Waste water

Depending on its source, wastewater has peculiar characteristics. Industrial wastewater with characteristics of municipal or domestic wastewater can be discharged together. Industrial wastewater may require some pretreatment if it has to be discharged with domestic wastewater. As you know different industries release certain specific types of toxic chemicals. Therefore, characteristics of wastewater vary from industry to industry and therefore would have different treatment processes. In general, the contaminants in wastewater are categorized into physical, chemical and biological.

Physical Properties

- **Electrical Conductivity:** It indicates the salt content
- **Total Dissolved Solids:** These solids comprise inorganic salts and small amounts of organic matter dissolved in water.
- **Suspended solids:** These solids comprises solid particles suspended (but not dissolved)in water

Chemical properties

- **Dissolved Oxygen:** It indicates the amount of oxygen in water

- **Biochemical oxygen demand (BOD):** It indicates the amount of oxygen required by aerobic microorganisms to decompose the organic matter in a specific time period.
- **Chemical oxygen demand:** It indicates the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.
- **NH4-N and NO3-N:** These show dissolved nitrogen (Ammonium and Nitrate, respectively).
- **Total Nitrogen:** It is a measurement of organically-bound ammonia nitrogen.
- **Total-P:** It reflects the amount of all forms of phosphorous in a sample.

Biological Properties

- **Total coliforms (TC):** It is encompassing faecal coliforms as well as common soil microorganisms, and is a broad indicator of possible water contamination.
- **fecal coliforms (FC):** It is an indicator of water contamination with faecal matter. The common lead indicator is the bacteria Escherichia coli or E. coli.
- Helminth analysis looks for worm eggs in the water

7.4 Wastewater Treatment

7.4.1. History of Wastewater treatment

Wastewater treatment is a quite new practice although drainage systems were built long before the 19th century. Before this time, “night soil” was placed in buckets along streets and workers emptied them into “honeywagon” tanks. This was sent to rural areas and disposed off over agricultural fields. In the 19th century, flush toilets led to an increase in the volume of waste for these agricultural lands. Due to this transporting challenge, cities began to use drainage and storm sewers to convey wastewater into water bodies. This process was against the recommendation of Edwin Chadwick in 1842 that “rain to the river and sewage to the soil”. The discharge of waste into water courses led to gross pollution and health problems for users.

In 1842, an English engineer named Lindley built the first “modern” sewerage system for wastewater carriage in Hamburg, Germany. The improvement of the Lindley system is

basically in improved materials and the inclusion of manholes and sewer appurtenances the Lindley principles are still upheld today. Between the late 1800s and early 1900s, various options were tried until in 1920, the processes we have today were tried. Its design was however empirical until midcentury. Centralized wastewater systems were designed and encouraged. The cost of wastewater treatment is borne by communities discharging into the plant.

7.4.2. Objectives of wastewater treatment

There are various objectives of the wastewater treatment which are given below:

1. Reduction of biodegradable organic substances: Organic substances such as carbon, nitrogen, phosphorus, sulfur in organic matter needs to be broken down by oxidation into gases which is either released or remains in solution.

2. Reduction of nutrient concentration: Nutrients such as nitrogen and phosphorous from wastewater in the environment enrich water bodies or render it eutrophic leading to the growth of algae. These plants deplete oxygen in water bodies and this hampers aquatic life.

3. Elimination of pathogens: As you know organisms that cause disease in plants, animals and humans are called pathogens. Examples of pathogens include bacteria (e.g. *Vibro cholerae*), viruses (e.g. *Enterovirus, hepatits* A & E virus), fungi (e.g. *Candida albicans*), protozoa (e.g *Entamoeba hystolitica, Giardia lamblia*) and helminthes (e.g. *Schistosoma mansoni, Asaris lumbricoides*).

4. Recycling and Reuse of water: Urbanization has also changed the agrarian nature of many areas. Population increase means more food has to be cultivated for the growing population and agriculture as we know is by far the largest user of available water which means that economic growth is placing new demands on available water supplies.

7.4.3 Types of Wastewater

There are various types of wastewater which are summarized in Fig-1

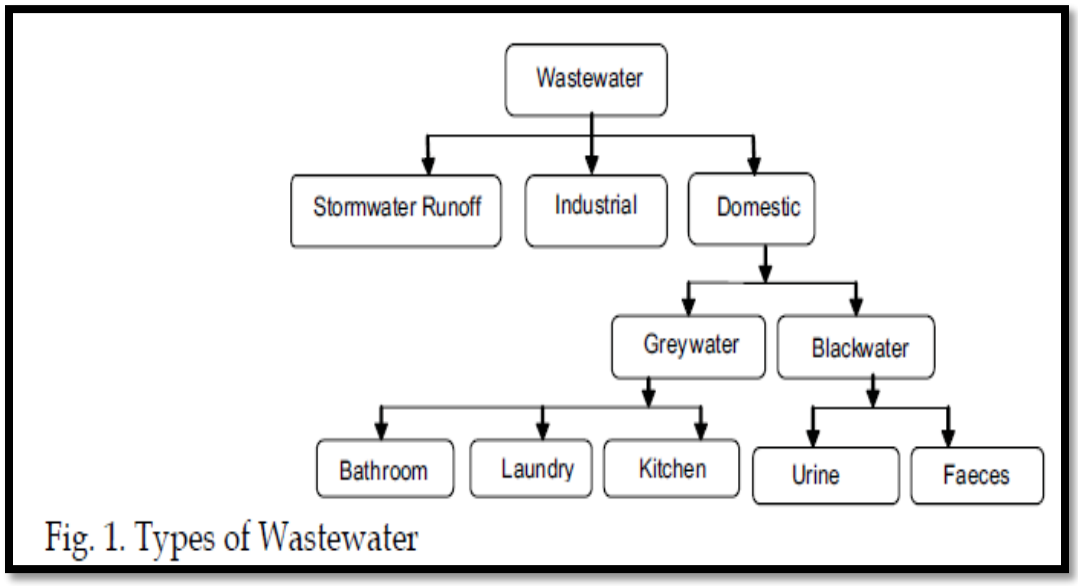


Fig. 1. Types of Wastewater

7.4.4 Primary Treament or Mechanical Treatment

Primary treatment is also called mechanical treatment. It removes settleable organic and inorganic solids by sedimentation and floating materials by skimming. Up to 50% of BOD, 70% of suspended solids and 65% of grease and oil can be removed after this treatment. Generally large sized particles are removed during this treatment. However, in this treatment, colloidal and dissolved constituents not removed. The effluent from primary sedimentation units is referred to as primary effluent (FAO, 2006). As sewage enters a plant for treatment, it flows through a screen, which removes large floating objects such as rags and sticks that might clog pipes or damage equipment. After sewage has been screened, it passes into a grit chamber, where cinders, sand, and small stones settle to the bottom. A grit chamber is particularly important in communities with combined sewer systems where sand or gravel may wash into sewers along with storm water. These solids are minute particles that can be removed from sewage in a sedimentation tank.

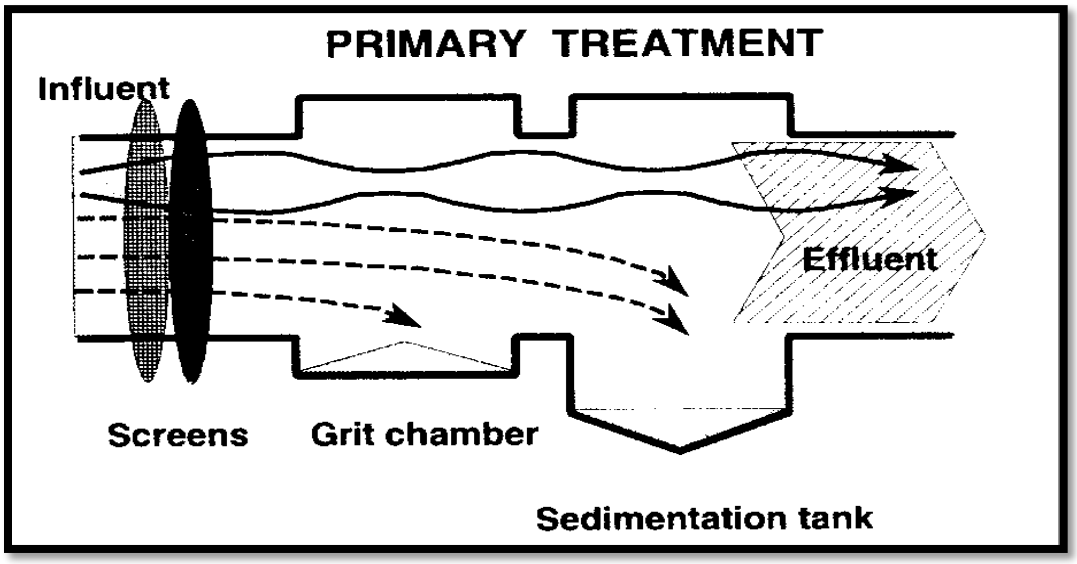


Fig-2: Showing primary treatment

7.4.5 Secondary Treatment or Biological treatment

Secondary treatment is the further treatment of primary effluent to remove residual organics and suspended solids. Also biodegradable dissolved and colloidal organic matter is removed using aerobic biological treatment processes. The removal of organic matter is when nitrogen compounds and phosphorus compounds and pathogenic microorganisms are removed. The treatment can be done mechanically like in trickling filters, activated sludge methods rotating biological contactors (RBC) or non-mechanically like in anaerobic treatment, oxidation ditches, stabilization ponds etc. The *secondary stage* of treatment removes about 85 percent of the organic matter in sewage by making use of the bacteria in it. The principal secondary treatment techniques used in secondary treatment are *the trickling filter* and the *activated sludge process.*

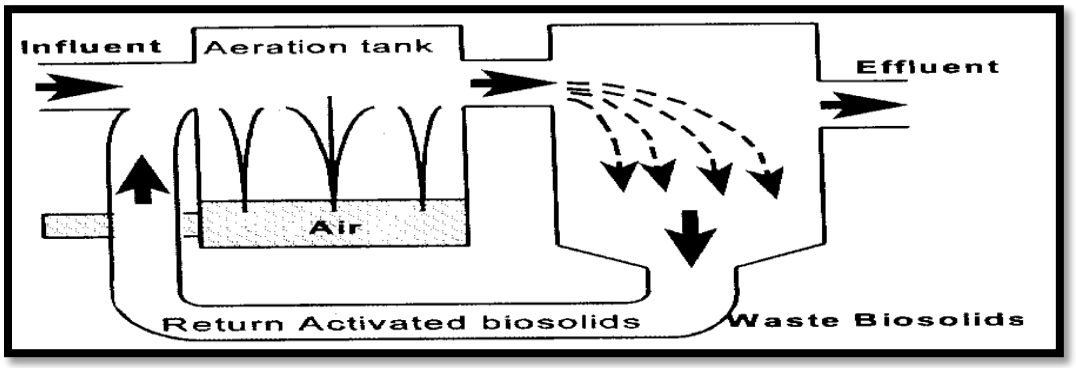


Fig-3: Showing secondary treatment

Biological unit operation has following steps.

Activated sludge process: In this process microorganisms (like: algae, bacteria, fungi, protozoans) are used to remove the organic matter. An activated sludge can also be referred to as sewage hat contains active microbes which help in the breakdown of organic matter. Activated sludge is the most effective of all the wastewater treatment systems. The microorganisms are composed of 70-90 % organic matter and 10-30 % inorganic matter. The concentrated biological solids are then recycled back to the aeration tank, to maintain a concentrated population of microorganisms to treat the wastewater. Because microorganisms are continuously produced in the system, a way must be provided to dissipate the excess biological solids produced. Activated process consists of an aeration tank, a means of stirring the mixture of fluid dispersed in the aeration tank, a means of separating the microorganisms from the treated water and a system of recycling some of the microorganisms back to the reactor. The activity of the microorganisms helps in the oxidation of the sewage organic matter into carbon dioxide and water. In the activated sludge, the carbonaceous organic matter of the wastewater provides the energy source for the production of new cells. The microbes convert the carbonaceous organic matter of the wastewater into cell tissue and oxidize end products that include CO2, SO4, NO3 and PO4. Most activated sludge processes are used to degrade carbonaceous biochemical oxygen demand (BOD). Bacteria, fungi, protozoa, rotifers and nematodes are commonly found in activated sludge, though all may not exist in any single system. Algae, because of their need for light rarely exist in mixed liquor. The overall reactions occurring in the activated sludge system are determined by the composite metabolism of all the microorganisms in the activated sludge. The metabolic process consists of the separate, yet simultaneously occurring reactions of synthesis and respiration.

Trickling filters: A trickling filter is a commonly used method of secondary wastewater treatment. It is made up of a filter bed that contains a highly permeable media (gravel or plastic material etc), which has a layer of microorganisms on the surface that leads to the formation of a slime layer. In a trickling filter system, the microorganisms are attached to the media in the bed and form a biofilm over it. As the wastewater passes through the media, the microorganisms consume and remove contaminants from the wastewater. In a trickling filter, the sewage is sprayed over the permeable media (bed of rocks, molded plastic, gravel

and ceramics etc). This treatment system removes 80 - 85% of the BOD so they are less efficient than activated sludge systems. Trickling filters consist of a septic tank, a clarifier and an application system. The septic tank helps with the removal of the solids present in the wastewater; the clarifier enables the biological materials to settle out of the wastewater and the application system assists in the distribution of the treated wastewater to the proper site. Trickling filters can be classified as high rate or low rate according to hydraulic or organic loading.

Membrane bioreactor: A membrane bioreactor is a combination of the biological degradation process of an activated sludge with a direct solid-liquid separation by membrane filtration through the use of micro or ultrafiltration membrane technology. The system allows for the complete physical retention of bacterial flocs and all suspended solids within the bioreactor. The advantages of a membrane bioreactor over other treatment systems are its high effluent quality, good disinfection capability, higher volumetric loading and less sludge production. A membrane bioreactor is a biological wastewater treatment process that uses membrane to replace the gravitational settling of the conventional activated sludge process for the solid liquid separation of sludge suspension. Membrane bioreactors are used to treat biologically active wastewater feeds from municipal or industrial sources Two MBR configurations could exist, internal/submerged and external/sidestream. In the submerged, the membranes are immersed in and integral to the biological reactor while in the external/sidestream, the membranes are a separate unit process that requires intermediate pumping steps. The membranes in membrane bioreactor system are made up of polymers or inorganic substances. They are made of several small pores, which can only be seen with the help of a microscope.

Stabilization pond: A wastewater stabilization pond is one of the most important natural methods for wastewater treatment. It is usually a shallow man-made pond that consists of single or several series of anaerobic, facultative or maturation ponds. In this system, the treatment of the wastewater starts from the anaerobic pond, which is designed for the removal of suspended solids and some other organic matter present. In the second stage, also known as the facultative pond, the remaining organic matter is removed through the activity of algae and the heterotrophic bacteria e.g *Arthrobacter* sp., *Rhodococcus* sp., *Pseudomonas* sp.. During the last stage, treatment is achieved through the maturation pond,

whose main function is the removal of pathogens and nutrients. The system is said to be a cost effective wastewater treatment system for the removal of pathogenic microorganisms. The treatment of the wastewater is gained through the use of natural disinfection mechanisms. Stabilization ponds are suitable for tropical and subtropical countries because of the intensity of sunlight and the temperature which aids the effectiveness of the removal processes. Anaerobic ponds are the smallest of the series of stabilization ponds processing, they are about 2-5m deep and receive high organic matter. This high organic matter produces strict anaerobic conditions meaning that dissolved oxygen is not present in the pond.

7.4.6 Tertiary or Chemical Treatment

Tertiary treatment or advance treatment is employed when specific wastewater constituents which cannot be removed by secondary treatment must be removed. Advance treatment removes significant amounts of nitrogen, phosphorus, heavy metals, biodegradable organics, bacteria and viruses. Two methods can be used effectively to filter secondary effluent—traditional sand (or similar media) filter and the newer membrane materials. Some filters have been improved, and both filters and membranes also remove helminths. The latest method is disk filtration which utilizes large disks of cloth media attached to rotating drums for filtration (FAO, 2006). At this stage, disinfection by the injection of Chlorine, Ozone and Ultra Violet (UV) irradiation can be done to make water meet current international standards for agricultural and urban re-use. Chemical unit operation has following steps:

Chemical precipitation: The dissolved inorganic components can be removed by adding an acid or alkali, by changing the temperature, or by precipitation as a solid. The precipitate can be removed by sedimentation, flotation, or other solid removal processes. Although chemicaL precipitation (coagulation, flocculation) is still implemented, it is highly recommended to substitute the chemical precipitation process by phytoremediation, where the trend is to ramp up the implementation of bioremediation and phytoremediation to reduce the use of chemicals, which is in line with the “Green Development”.

Neutralization: Neutralization is controlling the pH of the wastewater whether it is acidic or alkaline to keep the pH around. The lack of sufficient alkalinity will require the addition of a

base to adjust the pH to the acceptable range. Lime (CaO), calcium hydroxide ($Ca(OH)_2$), sodium hydroxide (NaOH), and sodium carbonate (Na_2CO_3), also known as soda ash, are the most common chemicals used to adjust the pH. The lack of sufficient acidity will require the addition of an acid to adjust the pH to the acceptable range. Sulfuric acid (H_2SO_4) and carbonic acid (H_2CO_3) are the most common chemicals used to adjust the pH.

Adsorption: Adsorption is a physical process where soluble molecules (adsorbate) are removed by attachment to the surface of a solid substrate (adsorbent). Adsorbents should have an extremely high specific surface area. Examples of adsorbents include activated alumina, clay colloids, hydroxides, resins, and activated carbon. The surface of the adsorbent should be free of adsorbate. Therefore, the adsorbent should be activated before use. A wide range of organic materials can be removed by adsorption, including detergents and toxic compounds. The most widely used adsorbent is activated carbon, which can be produced by pyrolytic carbonization of biomass. Activated carbon is the most implemented adsorbent

Disinfection: The disinfection of wastewater is the last treatment step of the tertiary treatment process. Disinfection is a chemical treatment process conducted by treating the effluent with the selected disinfectant to exterminate or at least inactivate the pathogens. The purpose of disinfection is the protection of the microbial wastewater quality. The ideal disinfectant should have bacterial toxicity, is inexpensive, not dangerous to handle, and should have reliable means of detecting the presence of a residual. The examples of disinfection agents are chlorine, ozone, ultraviolet radiation, chlorine dioxide, and bromine.

Ion exchange: Ion exchange is a reversible reaction in which a charged ion in a solution is exchanged with a similarly charged ion which is electro-statically attached to an immobile solid particle. The most common implementation of ion exchange method in wastewater treatment is for softening, where calcium and magnesium ions are exchanged with sodium. Typically, sodium is exchanged with cations in the solution. The bed is shut down when it becomes saturated with the exchanged ions, where it should be regenerated by passing a concentrated solution of sodium back through the bed.

7.4.7 Biodegradation of Toxic Pollutants

Organic pollutant is a major concern in the contemporary world. Organic pollutants may be treated through physical and chemical processes, but these processes are toxic and cause environmental problems hence, the biological approaches may be a suitable alternative towards bioremediation practices being not only cost effective but eco-friendly as well. Moreover, the final product happens to be less toxic as compared to other approaches. The microorganisms and plants (bioremediation) are used to remediate the polluted environments widely and is emerging as a promising and appealing area of environmental biotechnology. Apart from using the whole cell microorganisms, the use of their extracellular and/or cell-free enzymes has been advocated as an innovative technique to abate pollution. Employment of extracellular enzyme for the removal of pollutants has several advantages over using whole microbial cell.

7.5 Degradation of Organic Pollutants

Organic chemicals that are introduced into the environment are subjected to various physical, chemical, and biological processes which act in an interconnected way in environmental systems to determine the overall fate of the compound. The neutralization when done through chemical means, a huge amount of acid is used, which is neither economical, nor safe and poses serious health hazards. There are many processes for the degradation of organic pollutants. Some processes for degradation of organic pollutant are listed below:

1. Physical processes: Physical processes have been used for the degradation of organic pollutant from many decades, which may include various processes like photocatalytic degradation by using Ag-modified Zn GeO nanorods, TiO Bio-silica coated with amorphous manganese oxide etc. Decomposition of these organic pollutants via catalytic/ photocatalytic oxidation is considered to be the most efficient green method for organic waste management. Visible-light response semiconductors have attracted interest of many researchers as the efficient photo catalysts. There are many catalysts used for photo catalytic degradation of organic pollutants. TiO_2 used as a photo catalyst because of its low cost, chemical stability, non-toxicity. TiO is preferred because it is a promising photo-oxidation catalyst and has strong oxidizing ability of photo-induced holes.

2. Chemical Processes: The chemical methods for bioremediation include electrochemical de-halogenation of chlorinated benzenes, in this the chlorine is eliminated step by step from the highly chlorinated benzenes to yield less-chlorinated benzenes and finally transform to benzene.

3. Biological processes: Bioremediation of organic pollutant contaminated soil offers a cost-competitive treatment for many sites that are currently facing costly incineration or the extended liability of land disposal. In the Field, under conditions of full-scale site remediation, this technology has been shown to be cost effective. Different types of biological processes include bio-attenuation, bio-stimulation and bioaugmentation.

Bio-attenuation (Natural Attenuation): In this process, the pollutants are transformed to less harmful forms or immobilized forms. Such transformation and immobilization processes are largely due to biodegradation by microorganisms and to some extent by the reactions with naturally-occurring chemicals and sorption on the geologic media.

Bio-stimulation: Bio-stimulation is a process of decontamination of polluted soil in which growth of microbes is facilitated by modifying environment. The pace of microbial transformation of chemical pollutants heavily rely on supply and availability of nutrients like Carbon, Nitrogen, Potassium, available oxygen, optimum pH, redox potential and also on the type and concentration of organic pollutants. To stimulate microbial degradation, nutrients in the form of fertilizers, slow release and oleophilic are added.

Bio-augmentation: Bio-augmentation is the addition of bacterial cultures to speed up the rate of degradation of contaminants. The contaminated soil sediments contain microflora which are very well adapted to high concentration of organic pollutants. Microorganism isolated from contaminated soil sediments can be utilized for remediating soils freshly contaminated with hydrocarbons. Priming with 2% bio-remediated soil has been found to facilitate biodegradation of PAH components of soil treated with fuel oil.

7.5.1. Types of Bioprocess for Organic Pollutant Remediation

Bioremediation: It is technique in which microorganisms are used for the degradation of hazardous substances in soil, sediments, water or other contaminated matters. Certain species of bacteria fungi, algae and plants are used for bioremediation. **Bio augmentation**

is process in which microorganisms are imported to polluted site to enhance degradation of hazardous material.

On the basis of degradation procedure bioremediation may be of following types:

Biotransformation: It is alteration of contaminants in to less or non-hazardous substances.

Biodegradation: It is the breakdown of organic substances in smaller organic or inorganic molecules.

Mineralization: It is the complete biodegradation of organic material in to inorganic substances such as CO_2 or H_2O_4

On the basis of type of organisms used, bioremediation may be of following types:

1. **Bacterial Remediation:** It is the process of using bacteria to breakdown molecular contaminants like hydrocarbons in to simpler and safer components. **Deinococcus radiodurans** is genetically modified bacteria which can breakdown the heavy metals as well as toluene. **Geobacter sufurreducens** can turn uranium in to non-soluble form. Bacteria namely **Thermus brockianus** breaks down hydrogen peroxide 8000 times faster than current chemicals in use. **Alcaligenes eutrophus** another type of bacteria can degrade 2-4-D (Herbicide used in United States)
2. **Mycoremediation:** It is the process of using fungi to breakdown molecular contaminants in to simpler and safer components.

As you know bioremediation is the process where, in controlled conditions, microorganisms have been used for the removal of pollutants from contaminated sites. Bioremediation of toxic wastes can be categorized as in situ and ex situ bioremediation. In contaminated soil (e.g.) 5.0 g parathion/kg or more than 90% of parathion was destroyed within 3 weeks because of direct inoculation of Pseudomonads Pseudomonas stutzeri and Pseudomonas aeruginosa.

In-situ Bioremediation: In situ, process is applied on contaminated site without removing wastewater from its original position. Bioremediation which employs microorganisms to degrade toxic substances. In-situ processes may include Bio-attenuation, Bio-stimulation and Bioventing etc. Complete bioremediation of TNT (TriNitro Toluene) at laboratory-scale suggested that an initial anaerobic treatment was essential before the aerobic phase.

Rhizoremediation of TNT by colonizing ***Pseudomonas*** in plant rhizosphere (area of root). Transgenic plants (tobacco) that express the onr gene from ***Enterobacter cloacae*** have also been designed for bioremediation of TNT.

Ex-situ Bioremediation: In this type of bioremediation techniques, removal of contaminated material from the source (wastewater). Ex-situ processes include land farming, composting, phytoremediation and bio-restoration.

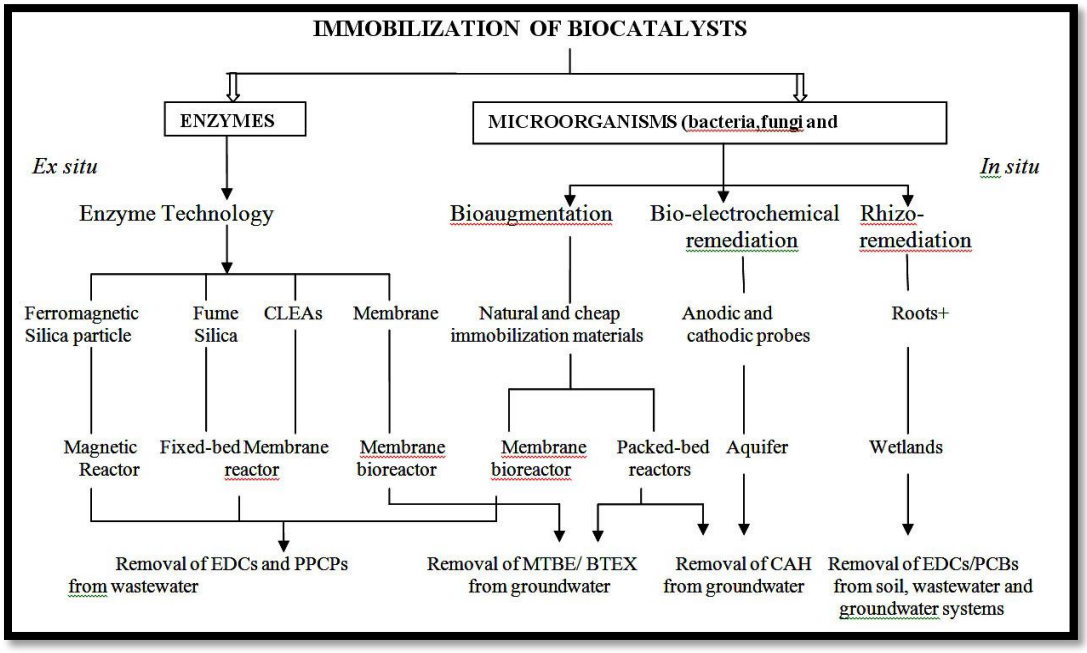


Fig-4: Showing immobilization of biocatalysts

7.5.2. Microbial Enzymes Involved in Wastewater Treatment

Microbial enzymes are catalysts of biological origin known to enhance the conversion of substrates into products by rendering favourable conditions by lowering the activation energy of the reaction. An enzyme may be a protein or a glycoprotein and consists of at least one polypeptide moiety.

1. Microbial Hydrolytic Enzymes (Hydrolases): Bacteria have been very well documented to play significant role in degradation of organic pollutants. Hydrolytic enzymes generally are involved in degradation of pollutants and reduction of their toxicity since they break down chemical linkages present in the toxic molecules. In the degradation of oil spill, organophosphate and carbamate insecticides, the use of hydrolytic enzymes has been

found very effective. DDT, an organochlorine insecticide and heptachlor are although stable in well-aerated soil, but can be degraded readily in anaerobic environments. Hemicellulase, cellulose and glycosidase are few important examples of such enzymes.

1.1 Microbial Lipases: Enzyme lipase is known to degrade lipids present in biomass of microbes, animals and plants. Microbial lipases have been deemed as more diverse due to their enumerable applications in industries. Reactions such as hydrolysis, inter-esterification, alcoholysis and aminolysis are generally catalyzed by microbial lipases. Lipase assay has been proposed as the most significant parameter for interpreting the extent of degradation of hydrocarbon in wastewater treatment.

1.2 Microbial Cellulases: Enzyme cellulases catalyze the conversion of waste cellulosic material into foods to cater the growing food demand of population. Cellulases are a mixture of several enzymes and three major groups of cellulases have been found to indulge in the hydrolysis of (1) endoglucanase (EG, endo- 1,4-D-glucanohydrolase) known to attack area of less crystallinity in the cellulose fiber, creating free chain ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-b-D-glucan cellobiohydrolase) with a potential to degrade the cellulose molecule further by eliminating cellobiose units from the free chain ends; (3) β-glucosidase which catalyzes further hydrolysis of cellobiose to glucose units.

1.3 Microbial Proteases: Microbial proteases catalyze the hydrolysis of proteinaceous substrate entering environment due to shedding and death of animals, moulting of appendages and also as byproduct of some industries like poultry, fishery and leather. Proteases hail to the group of enzymes known to hydrolyze peptide bonds in aqueous environment and synthesize them in non-aqueous environment. In food, leather, detergent and pharmaceutical industries, proteases have diverse applications. Proteases as ubiquitous enzymes have gained significant applications in biotechnology sector. Among the different proteases known, alkaline proteases have profound applications in industries like pharmaceutical, laundry detergents, leather processing proteinaceous waste bioremediation and food industry. The alkaline protease production by *Bacillus subtilis* and its possible application as a depilating agent have been reported. Researchers have reported the production of a salt tolerant protease from *Pseudomonas aeruginosa* BC1 and its application

in tannery saline wastewater treatment. However, these enzymes are mostly not significantly active at broad temperature and pH range.

2. Microbial Oxidoreductases: Oxidoreductases are known to catalyze the degradation of toxic organic compounds by enumerable bacteria and fungi and higher plants via oxidative coupling. Microorganisms may harness energy through energy-yielding biochemical reactions dominated by these enzymes to break chemical linkages and to consolidate the electron transfer from a reduced organic substrate (donor) to another chemical compound (acceptor). The oxidoreductases catalyze the process of humification of variety of compounds which are of phenolic in nature produced during degradation of lignin in soil sediments. The principal mechanism involved in the degradation of pollutants is the secretion of cluster of ligninolytic enzymes like laccase, lignin peroxidae and manganese peroxidase in soil by fungal mycelium. Filamentous fungi have large surface area to access soil pollutants more effectively than bacteria.

2.1. Microbial Oxygenases: Oxygenases belong to oxidoreductase class of enzymes and take part in oxidation of reduced substrates. On the basis of number of oxygen atoms used for oxygenation, oxygenases can be categorized into; (i) monooxygenases and (ii) dioxygenases. These enzymes either increase reactivity or water solubility of aromatic compounds or through cleavage of the aromatic ring hence, play important role in the metabolism of organic compounds. Haloginated compounds represent the largest group of environmental pollutants bearing widespread application as insecticides, herbicides, hydraulic, fungicides, heat transfer fluids, intermediates for chemical synthesis and plasticizers.

2.2 Monooxygenases: Enzyme Monooxygenases act by incorporating one atom of the oxygen molecule into the substrate. Monooxygenases consists of a versatile super family of enzymes catalyzing oxidative reactions of substrates ranging from alkanes to complex endogenous molecules such as steroids and fatty acids.

Monooxygenases catalyze a wide spectrum of reactions such as dehalogenation, desulfurization, ammonification, denitrification, biotransformation, hydroxylation and biodegradation of various aromatic and aliphatic compounds. Oxygenases play important

role in degradation of hydrocarbon such as substituted methanes, alkanes, cycloalkanes, alkenes, haloalkenes, ethers, and aromatic and heterocyclic hydrocarbons.

Microbial Dioxygenases: Dioxygenases are multicomponent enzyme systems reported to incorporate molecular oxygen into substrates. These dioxygenases catalyze the oxygenation of diverse substrates. In the beginning, Dioxygenases were known to catalyze the oxidation of aromatic compounds and, hence, have applications in environmental remediation.

2.4. Microbial Laccases: Laccases are a family of multicopper oxidases secreted by some plants, fungi, insects and bacteria that have the potential to catalyze the oxidation of a wide range of reduced phenolic and aromatic substrates. Laccase may be inhibited by variety of reagents such as halides (excluding iodide), azide, cyanide and hydroxide. It has been reported that different laccases have differential tolerance toward inhibition by halides, indicating differential halide accessibility. Laccase production has been found sensitive to the nitrogen concentration in fungi. High nitrogen levels are usually essential to obtain greater amounts of laccase.

2.5. Microbial Peroxidases: Peroxidases are ubiquitous enzymes bearing boundless potential to catalyze oxidation of lignin and other phenolic compounds using hydrogen peroxide in the presence of a mediator. Heme and non-heme proteins may be present in peroxidases.

2.5.1. Microbial Lignin Peroxidases: White rot fungus during secondary metabolism secrete lignin peroxidases which are heme proteins. Lignin peroxidases have been shown to oxidize halogenated phenolic compounds, polycyclic aromatic compounds and other aromatic compounds followed by a series of non-enzymatic reactions.

2.5.2. Microbial Manganese Peroxidases: MnP is an heme containing extracellular enzyme produced by the lignin-degrading basidiomycetes fungus, which catalyzes oxidation of Mn^{2+} to the oxidant Mn^{3+} in a series of reactions. Mn^{2+} not only stimulates the production of MnP but also acts as a substrate for MnP. The Mn^{3+}, produced by MnP has been found to act as a mediator for the oxidation of various phenolic compounds.

2.5.3. Microbial Versatile Peroxidases: Versatile Peroxidase enzymes have potential to catalyze the oxidation of Mn^{2+}, methoxy benzenes, phenolic aromatic substrates like that of

MnP, LiP, and horseradish peroxidase. It has peculiar broad substrate specificity and has the potential to oxidize the substrates in the absence of manganese as compared to other peroxidases.

7.6 Microbiology of biological treatment process

The quality of wastewater effluents is responsible for the degradation of receiving water bodies, such as lakes, rivers, streams. The two main processes for the removal of impurities from wastewater influents are chemical and biological treatment but due to some drawbacks of the chemical treatment, biological treatment is now employed. Microorganisms are of major importance in industrial wastewater treatment, agricultural and aquaculture. They reside in the sediment and other substrates, and in the water of aquaculture facilities. Microorganisms may have positive or negative effects on the outcome of aquaculture operations. The major microbial populations found in wastewater treatment systems are bacteria, protozoa, viruses, fungi, algae and helminthes.

Bacteria: In wastewater treatment systems, bacteria play vital role in the conversion of organic matter present to less complex compounds. In terms of size, bacteria range from 0.2- 2.0 µm in diameter and are responsible for most of the wastewater treatment in septic tanks. Though not all bacteria are harmful, a number of them cause water-related diseases in human and animals. Some of these diseases include cholera, dysentery, typhoid fever, salmonellosis and gastroenteritis. Certain strains of ***Pseudomonas*** and ***Escherichia coli*** which may affect the newborn are potential sources of this disease. These strains of microbes have also been implicated in gastrointestinal disease outbreaks. Bacteria are of the greatest numerical importance in wastewater treatment systems. The majority is facultative living in either the presence or absence of oxygen. Although both heterotrophic and autotrophic bacteria are found in wastewater treatment systems the predominant ones are the heterotrophic bacteria. Some important bacteria genera that are found in wastewater treatment systems are ***Achromobacter, Alcaligenes, Arthrobacter, Citromonas, Flavobacterium, Pseudomonas, Zoogloe*** and ***Acinetobacter***. In wastewater treatment systems, bacteria are responsible for the stabilization of influent wastes. The majority of the bacteria are known to form floc particles. The floc particles are clusters of bacteria that break down waste. Also, the floc particles also serve as sites on which waste can be absorbed and

broken down. Filamentous bacteria form, which trichomes or filaments provide a backbone for the floc particles, allowing the particles to grow in size and withstand the shearing action in the treatment process. When filamentous bacteria are present in excessive numbers or length, they often cause solid/liquid separation or settleability problems. Furthermore, bacteria are the most common microbial pollutants in wastewater. The presence of pathogenic bacteria can be indicated using the tests for total and faecal coliforms. Conventionally, the detection of faecal coliform is generally accepted as a reliable indicator of faecal contamination. *Escherichia coli* is also regarded as a good and reliable indicator for faecal pollution from animal and human sources since it is known not to last for long periods outside the faecal environment. The tests for total and faecal coliforms can be carried out, using either the traditional or enzymatic methods.

Protozoa: Protozoa are microscopic, unicellular organisms that are also found in freshwater and also in the wastewater treatment systems. They perform many beneficial functions in the treatment process, including the clarification of the secondary effluent through the removal of bacteria, flocculation of suspended material and as bioindicators of the health of the sludge. The protozoa that inhabit the wastewater treatment systems are capable of movement in at least one stage of their development. They are 10 times bigger than bacteria; they are unicellular organisms with membrane enclosed organelles. Protozoa prey on pathogenic bacteria which make it have an advantage in wastewater. They can be classified into five groups depending on their mode of locomotion, which are the free swimming ciliates, crawling ciliates, stalked and sessile ciliates, flagellates and amoeboid. In wastewater treatment systems, protozoa are useful biological indicators of the condition of the systems.

Some ciliates, however, are predators of other ciliates or are omnivorous, feeding on a variety of organisms including small ciliates, flagellates and dispersed bacteria. All bacterivorous ciliates rely on ciliary currents to force suspended bacteria to the oral region. Ciliated protozoa are numerically the most common species of protozoa in activated sludge, but flagellated protozoa and amoebae may also be present. The species of ciliated protozoa most commonly observed in wastewater treatment processes include ***Aspidiscacostata, Carchesiumpolypinum, Chilodonellauncinata, Operculariacoarcta, Opercullariamicrodiscum, Trachelophyllumpusillum, Vorticella convallaria*** and ***Vorticella microstoma***. There is indication that the free-swimming ciliates such as Litonotus

sp. and Paramecium sp., which have cilia on all their body surfaces are typically found suspended or swimming freely in the bulk solution. On the other hand, the crawling ciliates, such as Aspidisca sp. and Euplotes sp. possess cilia only on their ventral or belly surface where the mouth opening is located. The crawling ciliates are usually found on floc particles while the stalked ciliates, such as Carchesium sp. and Vorticella sp., possess their cilia around the mouth opening only and are attached to floc particles. They have enlarged anterior portion and a slender posterior portion. The beating of the cilia and the springing action of the stalk produce a water vortex that draws dispersed bacteria into the mouth opening. In wastewater systems, two types of amoebae are predominant, naked, such as Actinophyrs sp., Mayorella sp. and Thecamoeba sp. and the shelled amoebae or testate amoebae, e.g. Cyclopyxis sp. The naked amoeba lack any protective covering while shelled amoebae possess a protective covering that consists of calcified material. flagellated protozoa are oval in shape and possess one or more whip-like flagella.

Viruses: Viruses are also found in wastewaters, particularly human viruses that are excreted in large quantities in faeces. Viruses that are native to animals and plants exist in smaller quantities in wastewater, although bacterial viruses may also be present. They are the causative agents of some water-related infections in humans, such as gastrointestinal and respiratory infections, conjunctivitis and meningitis. It is reported that a majority of waterborne diseases due to unidentified sources were caused by enteric viruses. They are very notorious and persistent when present in wastewater and can remain a viable source of infection for months after their entry into the wastewater.

Fungi: Fungi are also part of the microorganisms found in wastewater treatment systems. Fungi are multicellular organisms that are also constituents of the activated sludge. Under certain environmental conditions in a mixed culture, they metabolize organic compounds and can successfully compete with bacteria. The most common sewage fungus organisms are ***Sphaerotilus natans*** and ***Zoogloea sp***. A number of filamentous fungi are found naturally in wastewater treatment systems as spores or vegetative cells, although they can also metabolize organic substances. A number of fungi species, such as ***Aspergillus, Penicillium, Fusarium, Absidia*** and a host of others have been implicated in the removal of carbon and nutrient sources in wastewater.

Algae: Algae can be found in wastewater because they are able to use solar energy for photosynthesis as well as nitrogen and phosphorus for their growth leading to eutrophication. Some types of algae that can be found in wastewater include ***Euglena sp.,*** ***Chlamydomonas sp.,*** and ***Oscillatoria sp.*** Algae are significant organisms for biological purification of wastewater because they can be able to accumulate plant nutrients, heavy metals, and pesticides, organic and inorganic toxic substances. The use of microalgae in biological wastewater treatment has gained a lot of importance over the years. High rate algal pond is shallow and equipped with mechanical aeration and mixing by means of paddle wheels, 90% of BOD and 80% of nitrogen and phosphorus are treated in high rate algal ponds.

Helminths: Nematodes are aquatic animals present in fresh, brackish and salt waters and wet or humid soil worldwide. Freshwater nematodes can be present in sand filters and aerobic treatment plants. They are present in large numbers in secondary wastewater effluents, biofilters and biological contractors. Freshwater nematodes inhabit freshwater below the water table with species utilizing oxygen dissolved in the fresh water. Nematodes are part of the ecosystem, serving as food for small invertebrates. A lack of nematode activity can be one of the bio-indicators of a toxic condition that may be developing in the treatment process.

Summary

In this unit we have discussed various aspects of wastewater treatment. So far you have learnt that:

- Wastewater is domestic effluent consisting of black-water (excreta, urine and faecal sludge) and grey-water (kitchen and bathing wastewater);
- Water from commercial establishments and institutions, including hospitals;
- Industrial effluent, storm-water and other urban run-off;
- Agricultural, horticultural and aquaculture effluent, either dissolved or as suspended matter"
- Stormwater runoff: it is water from streets, open yard etc after a rainfall event which run through drains or sewers.
- Industrial wastewater: it is liquid waste from industrial establishments such as factories, production units etc.

- Domestic wastewater : it is also known as municipal wastewater is basically wastewater from houses, hotels and institutions. Further it can be categorized into grey-water and black-water.
- Grey-water: it is also known as sullage is liquid waste from washrooms, laundries, kitchens which does not contain human or animal excreta.
- Black-water: it is wastewater generated in toilets. Black-water may also contain some flush water besides urine and faeces. Urine and faeces together is sometimes referred to as night soil.
- Sewage: it is the term used for black-water if it ends up in a sewerage system.
- Physical properties of wastewater include electrical conductivity, total dissolved solids, suspended solids, chemical properties of wastewater include dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, nh4-n and no3-n and biological properties include: total coliforms, faecal coliforms and helminth
- Objectives of wastewater treatment are :
- Reduction of biodegradable organic substances, reduction of nutrient concentration, elimination of pathogens, recycling and reuse of water
- Primary treatment is also called mechanical treatment. It removes settleable organic and inorganic solids by sedimentation and floating materials by skimming. Up to 50% of bod, 70% of suspended solids and 65% of grease and oil can be removed after this treatment. Generally large sized particles are removed during this treatment. Secondary treatment is the further treatment of primary effluent to remove residual organics and suspended solids. Also biodegradable dissolved and colloidal organic matter is removed using aerobic biological treatment processes.
- Tertiary treatment or advance treatment is employed when specific wastewater constituents which cannot be removed by secondary treatment must be removed.
- Organic pollutant is a major concern in the contemporary world. Organic pollutants may be treated
- Physical processes: physical processes have been used for the degradation of organic pollutant from many decades, which may include various processes like photocatalytic degradation by using ag-modified zn geo nanorods, tio bio-silica coated with amorphous manganese oxide etc. Decomposition of these organic pollutants via catalytic/ photocatalytic oxidation is considered to be the most efficient green method for organic waste management. Visible-light response semiconductors have attracted interest of many researchers as the efficient photo catalysts. There are many catalysts used for photo catalytic degradation of organic pollutants. Tio_2 used as a photo catalyst because of its low cost, chemical stability, non-toxicity. Tio is preferred because it is a promising photo-oxidation catalyst and has strong oxidizing ability of photo-induced holes.
- Chemical processes: the chemical methods for bioremediation include electrochemical dehalogenation of chlorinated benzenes, in this the chlorine is eliminated step by step from the highly chlorinated benzenes to yield less-chlorinated benzenes and finally transform to benzene.

- Bioremediation: it is technique in which microorganisms are used for the degradation of hazardous substances in soil, sediments, water or other contaminated matters. Certain species of bacteria fungi, algae and plants are used for bioremediation. Bioaugmentation is process in which microorganisms are imported to polluted site to enhance degradation of hazardous material.
- Microbial enzymes involved in wastewater treatment are microbial hydrolytic enzymes (hydrolases, microbial lipases, microbial cellulases, microbial proteases, microbial oxidoreductases, microbial oxygenases, monooxygenases, microbial dioxygenases, microbial laccases, microbial peroxidases, microbial lignin peroxidases, microbial manganese peroxidases, microbial versatile peroxidases
- the major microbial populations found in wastewater treatment systems are bacteria, protozoa, viruses, fungi, algae and helminthes.
- Some important bacteria genera that are found in wastewater treatment systems are achromobacter, alcaligenes, arthrobacter, citromonas, flavobacterium, pseudomonas, zoogloe and acinetobacter.
- These groups are the free-swimming ciliates, crawling ciliates and stalked/sessile ciliates, flagellates and amoebae.
- The species of ciliated protozoa most commonly observed in wastewater treatment processes include aspidiscacostata, carchesiumpolypinum, chilodonellauncinata, operculariacoarcta, opercullariamicrodiscum, trachelophyllumpusillum, vorticella convallaria and vorticella microstoma.
- The most common sewage fungus organisms are sphaerotilus natans and zoogloea sp. A number of filamentous fungi are found naturally in wastewater treatment systems as spores or vegetative cells, although they can also metabolize organic substances. A number of fungi species, such as aspergillus, penicillium, fusarium, absidia and a host of others have been implicated in the removal of carbon and nutrient sources in wastewater.
- Some types of algae that can be found in wastewater include euglena sp., chlamydomonas sp., and oscillatoria sp.

Terminal Questions

1 (a) Fill in the blank spaces with appropriate words.

Wastewater treatment is a quite new practice although drainage systems were built long before the …….. century. Before this time, ……..was placed in buckets along streets and workers emptied them into “honeywagon” tanks. This was sent to rural areas and

disposed off over agricultural fields. In the 19th century, flush toilets led to an increase in the volume of waste for these agricultural lands. Due to this transporting challenge, cities began to use drainage and storm sewers to convey wastewater into water bodies. This process was against the recommendation of …….. ……..in 1842 that …….. …….. ……. ……. ……. ……. ……. ……. ……. …….The discharge of waste into water courses led to gross pollution and health problems for users.

In……., an English engineer named ……built the first ……..sewerage system for wastewater carriage in…… ……... The improvement of the Lindley system is basically in improved materials and the inclusion of manholes and sewer appurtenances the Lindley principles are still upheld today. Between the late 1800s and early 1900s, various options were tried until in 1920, the processes we have today were tried. Its design was however empirical until midcentury. Centralized wastewater systems were designed and encouraged. The cost of wastewater treatment is borne by communities discharging into the plant.

2 (a) Define the wastewater.

(b) Describe primary treatment of wastewater

3. (a) Describe the Secondary treatment of wastewater.

(b) What do you understand by tertiary treatment of wastewater? Explain

4. a) Give the process of biodegradation of toxic pollutants.

5. (a) Discuss bioremediation.

(b) Write about the enzymes involved in biodegradation of pollutants.

6 (a) Fill the blank spaces with appropriate words.

Primary treatment is also called…… ……**.** It removes settleable organic and …….solids by sedimentation and floating materials (scum) by skimming. Up to……. ……, ……of suspended solids and …….of grease and oil can be removed at this stage. Some organic nitrogen, organic phosphorus, and heavy metals are also removed. Colloidal and dissolved constituents are however not removed at this stage. The effluent from primary sedimentation units is referred to as primary effluent…….. As sewage enters a plant for treatment, it flows through a screen, which removes large floating objects such as rags and sticks that might clog pipes or damage equipment. After sewage has been screened, it passes into a…… ……, where cinders, sand, and small stones settle to the

bottom. A …… ……..is particularly important in communities with combined sewer systems where sand or gravel may wash into sewers along with storm water. After screening is completed and grit has been removed, sewage still contains organic and inorganic matter along with other suspended solids. These solids are minute particles that can be removed from sewage in a……. …….. When the speed of the flow through one of these tanks is reduced, the suspended solids will gradually sink to the bottom, where they form a mass of solids called *raw primary biosolids formerly sludge).*

(b) Primary treatment of wastewater is also known as (Biological treatment/Chemical Treatment/Mechanical treatment/None of the above)

(c) Chemical Treatment is also known as (Primary treatment/secondary treatment/Tertiary treatment**)**

(d) Grit chamber is used during (Primary treatment/secondary treatment/Tertiary treatment**)**

(e) Discuss the microbiology of biological treatment.

7. (a) Discuss the properties and composition of wastewater

(b) Describe the objectives of wastewater treatment

Answers

1 (a) 19th, “night soil”, Edwin Chadwick, “rain to the river and sewage to the soil”, 1842, Lindley, “modern”, Hamburg, Germany

2 (a) see section 7.2.

(b) See section 7.4.4

3 (a) See section 7.4.5.

(b) See section 7.4.6

4 (a) See section 7.5.

5 (a) See the section 7.5.1

(b) see the section 7.5.2

6 (a) mechanical treatment, 50% of BOD, 70%, FAO, 2006, grit chamber, grit chamber, sedimentation tank,

(b) Mechanical Treatment

(c) Tertiary Treatment

(d) Primary Treatment

(e) See the section 7.6.

7 (a) See the section 7.3.

(b) See the section 7.4.2

Unit 8 Waste Management

Unit Structure

8.0 Learning Objectives

After studying this unit you will be able to understand:

- Bioremediation
- Technologies for bioremediation
- Aerobic and anaerobic Biodegradation
- Energy generation from waste
- Health aspects and toxicity

8.1 Introduction

The global environment is under great stress due to urbanization and industrialization as well as population pressure on the limited natural resources. The problems are compounded by drastic changes that have been taking place in the life style and habits of people. The environmental problems are diverse and sometimes specific with reference to time and space. The nature and the magnitude of the problems are ever changing, bringing new challenges and creating a constant need for evolving newer and more appropriate technologies. In this context, biotechnology has tremendous potential to cater to the need and holds hope for environmental protection and management. While some application such as for bioremediation are direct applications of biotechnology there are many which are indirectly beneficial for the environment.

8.2 Bioremediation

Bioremediation is made of Greek words “Bio meaning Life” and latin word “Remedian meaning Restoring balance”. Thus, ‘Bioremediation’ is the process of using biological organisms to solve an environmental problem such as contaminated soil or groundwater”. It is the cleaning up technology that uses naturally occurring organisms to degrade or transform various hazardous chemicals to less harmful or nontoxic compounds”. Bioremediation is also known as biotreatment, bioreclamation, and biorestoration. It is limited to those compounds that are biodegradable.

Bioremediation of a contaminated site typically works in one of two ways:

(1) In one case / microbes eat and digest organic substances as their food for nutrients and energy. When microbes completely digest chemicals they change them into water and harmless gases with an end product of clean soil, CO2, and H2O, and cell material.

(2) In another case microbes degrade hazardous organic substances into inert products.

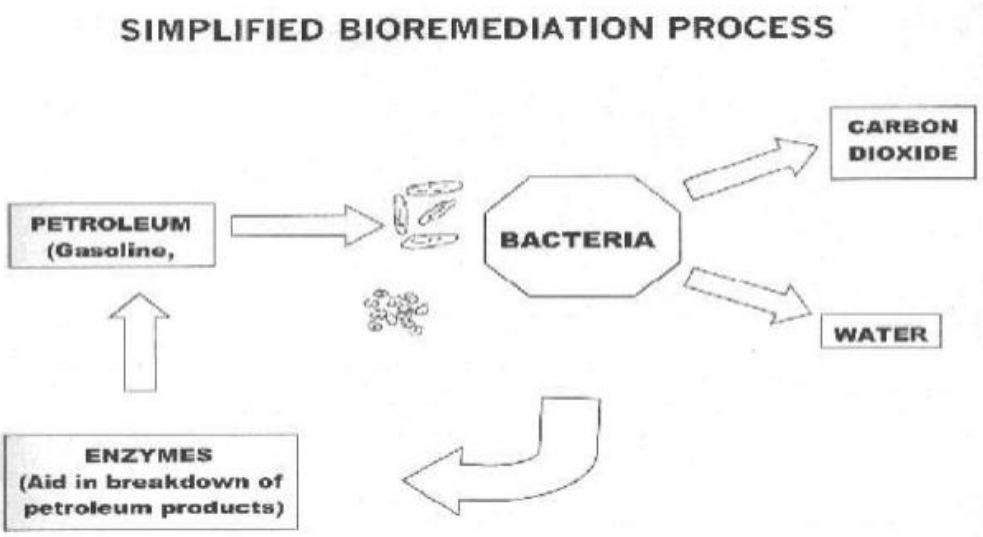


Fig 1: Bioremediation process

The problems of environment can be classified into the following subheads as most of the problems can be traced to one or more of the following either directly or indirectly.

1. Waste generation

a. Domestic waste (sewage, waste water, kitchen waste)

b. Industrial waste (by-products, effluents)

c. Agricultural waste (materials other than produce or materials left after separation/ extraction of produce)

d. Food waste (food processing industries)

e. Wood waste

2. Use of chemicals for various purposes

a. Insecticides

b. Pesticides

c. Chemical fertilizers

d. Toxic products and by-products from chemical industries

3. Over-exploitation of natural resources and loss

a. By conversion to non-recoverable forms, interference with biogeochemical cycles.

Waste generation is a side effect of consumption and production activities and tends to increase with economic advance. What is of concern is the increased presence of toxic chemicals such as halogen aliphatics, aromatics, polychlorinated biphenyls, polycyclic hydrocarbons etc. in the natural environment. These pollutants may reach air, water or soil and affect environment in several ways threatening the self-regulating capacity of the biosphere. They may be present in high levels at the point of discharge or may remain low but can be highly toxic. The underground water sources are increasingly becoming contaminated. Some substances may reach environment in small concentrations but may lead subjected to bio-magnifications wherein their concentrations increase as they pass through food chain e.g. DDT. A number of reasons including our dependence on industries for economy, the extent of damage involved, as well as the extent of the area involved makes it difficult to totally prevent such pollution or adopt classical methods to remove the pollutants effectively and economically.

There are three main approaches in dealing with contaminated sites:

A. Identification of the problem of the problem

B. Assessment of the nature and degree of the hazard

C. Choice of remedial action

Significance of microorganisms in Bioremediation: Microorganisms play a pivotal role in biotechnology, especially in dealing with environment though we cannot exclude the use of higher organisms, especially plant and animal cell cultures in biotechnology. Microorganisms are primary fixers of photosynthetic energy and they act as systems for bringing about chemical changes in almost all types of natural and most synthetic organic molecules.

Exploitation of the degradative capacities of microorganisms is the fundamental basis of organic pollutant bioremediation. Such microorganism that perform the function of bioremediation is known as **bioremediators**

The development and application of cost effective, efficient and environmentally sound

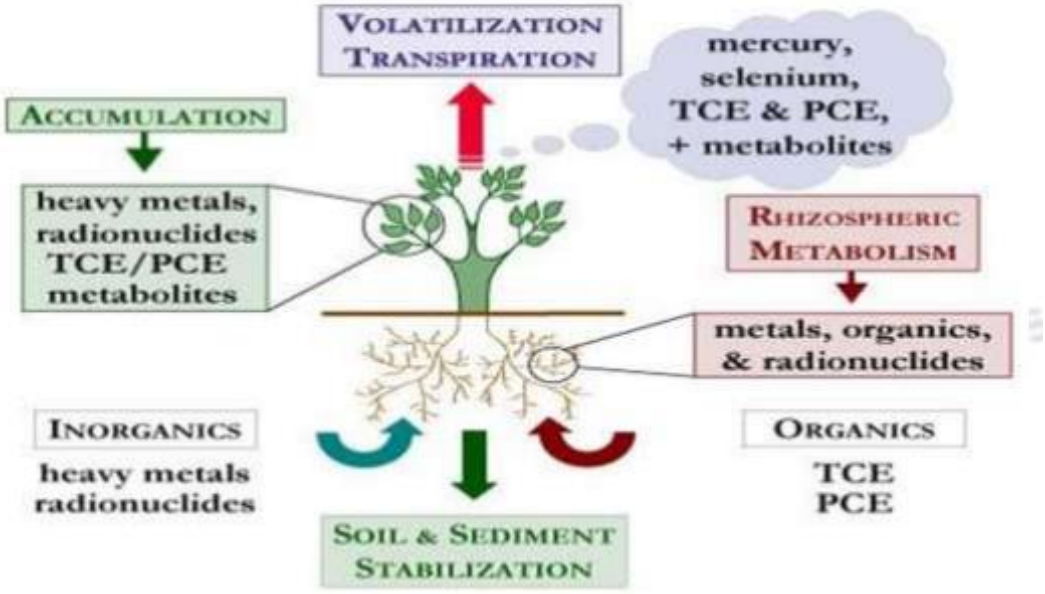


Fig 2: Concept of Bioremediation

bioremediation technologies depends on the integration of many different disciplines as well as on advances in our understanding of the physicochemical, biological, and ecological factors that govern microbial degradation of organic contaminants both *in situ* and *ex situ*.

Bioremediation is useful for the complete destruction of wide variety of contaminants. It can prove to be less expensive than other technologies that are used for cleaning up of hazardous waste.

8.3 Technologies for Bioremediation

Bioremediation treatment technologies have been broadly divided into two categories based on whether biodegradation is stimulated *in situ* i.e within the affected environment (soil, sediment, surface water, ground water etc.) or *ex situ* i.e under contained conditions (compost pits, bioreactors etc.). The latter usually involves removal of the contaminated material to the place of treatment with increased chances of exposure of the persons involved.

8.3.1 *In-situ* Bioremediation

In any natural setting, there is an existing microbial population which is ultimately responsible for the stabilization and treatment of waste products containing organic matter. This microbial population consists of a variety of microorganisms which invariably have minimum oxygen and nutrient requirements. Many of the problems encountered during handling of waste materials can be directly related to insufficient quantities of the two limiting factors namely oxygen and available nutrients. When the system is oxygen deficient, oxygen requiring bacteria do not thrive well and they give way to anaerobic bacteria. The natural respiration process of anaerobic bacteria can lead to generation of volatile organic compounds that are particularly noxious. The natural respiration process of these anaerobes involves the degradation of organic material into carbon dioxide and water. Many treatment systems attempt to encourage the proliferation of these anaerobes in order to breakdown waste material without generating odours.

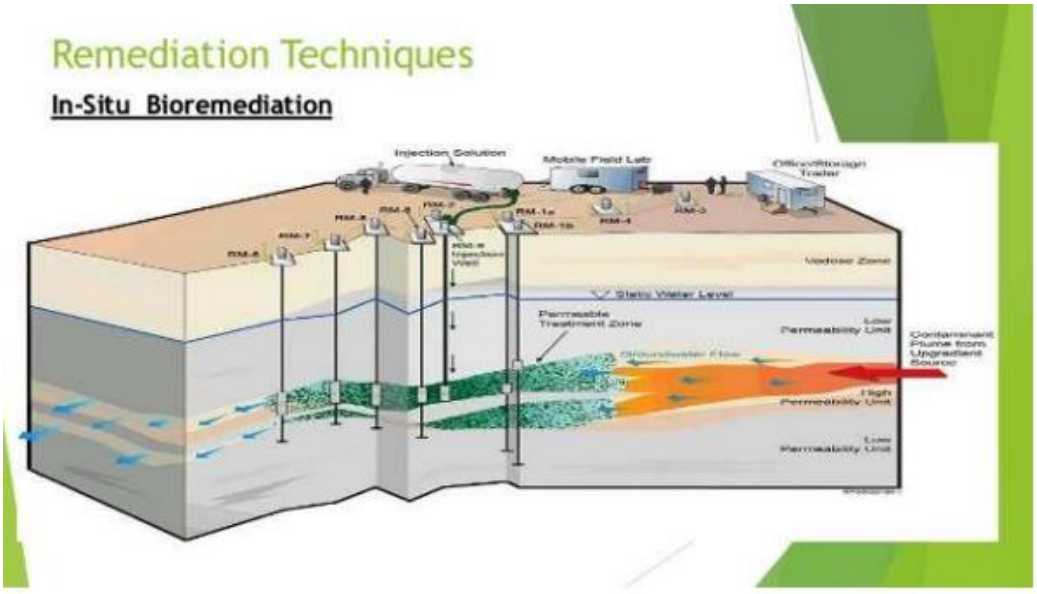


Fig 3: In situ Bioremediation

Availability of nutrients to the microbial populations within the waste material is another limiting factor determining the rate of breakdown of waste. They may be insufficient or may not be readily available. The nutrients which may act as limiting factors for the growth of microorganisms are amino acids, purines, pyrimidines and vitamins. These substances are

often referred to as growth factors. Thus, *in situ* bioremediation involves stimulation of the degradative activities of endogenous microbial populations by the provision of nutrients and or external electron receptors. Another way of enhancing microbial action is by the introduction of competent, exogenous microbes with or without nutritional enrichment. This procedure which is used to enhance microbial activity is called **Bioaugmentaton**.

The addition of nutrients has also been employed for treating contaminated soils. For soil bioremediation, apart from the addition of nutrients, oxygen is provided through oxygen saturated water, air sparging or Bioventing. Water containing water oxygen and nutrients is added to the surface of the contaminated area and allowed to percolate through the soil and mix with the groundwater.

Hydrogen peroxide has also been widely used as an alternate oxygen source in the percolation technology. In case of air **sparging**, air is injected into the water saturated zone of soils and sediments. Whereas **Bioventing** involves supply of air to soil to be treated using

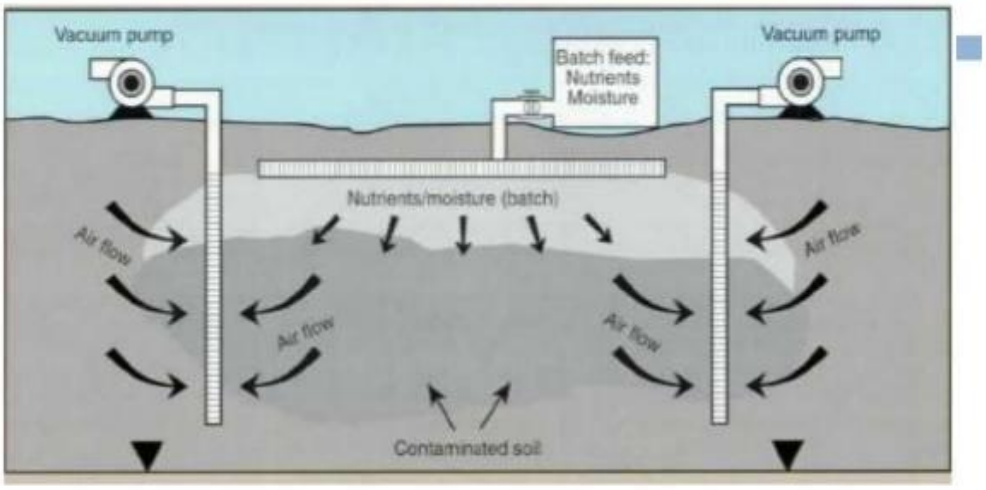


Fig 4: Bioventing

a combination of pumps and blowers. Using these devices, vacuum is applied to the target area, while continuously injecting low volumes of air. This procedure of bioventing is aimed at stimulating *in situ* aerobic degradation of contaminants, for its successful functioning, nutrients are often required to be added and adequate water levels must be present in the unsaturated zone to support microbial degradative activity.

Bioventing has been extensively used for *in situ* remediation of unsaturated soils contaminated by petroleum hydrocarbons. Often bioventing is combined with another technique called **soil vapour extraction** for faster remediation of highly contaminated sites.

8.3.2 *Ex-situ* Bioremediation

These techniques are usually aerobic and involve treatment of contaminated soils or slurry phase systems. Solid phase systems include **land farming** (soil treatment units), **compost heaps** and **engineered biopiles**.

Land farming involves plowing, tilling, and raking of the contaminated soil on site together with application of water, nutrients and microbial inocula if required. Care should be taken to prevent contamination of groundwater by leachate from the tilled zone.

Above ground composting techniques involve physical removal of the contaminated soil or sediment to specially constructed platforms, lined pads, or compost sheds. Compost heaps consist of contaminated soil or sediment supplemented with composting material (straw, wood chips, etc.) in order to enhance water and air holding capacity and improve physical handling properties. Periodic mixing or turning is applied in order to ensure to adequate aeration.

In engineered biopiles, aeration is constantly supplied via a network of piles. Nutrients and microbial inocula are added, if required to compost heaps and biopiles.

Composting techniques are used for remediation of highly contaminated sites and have proved successful for military sites contaminated with explosives such as TNT, RDX and tetryl.

Slurry phase bioremediation is a batch treatment technique in which excavated soils or sediments are mixed with water and treated in reactor vessels or in contained ponds or lagoons.

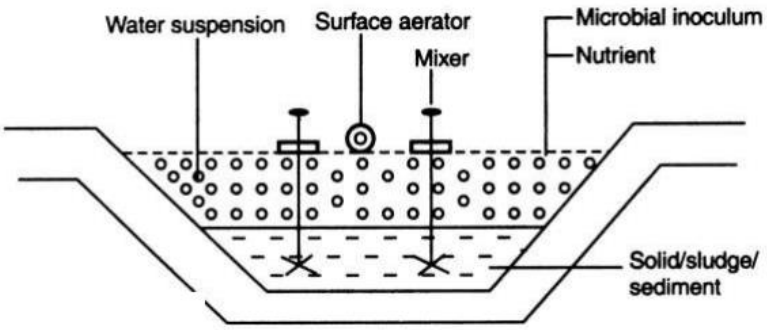


Fig 5: Ex situ Bioremediation

8.3.3 Factors affecting Bioremediation

A number of environmental factors influence in situ bioremediation rates. Some of them can be controlled or modified while some are difficult to control or modify.

1. Hydrogen ion concentration (pH): Majority of bacteria exhibit growth optima at or near neutral pH. Many soils are acidic throughput the world. Treatment commonly known as liming involves addition of finely ground agricultural limestone, calcium carbonate, calcium hydroxide, magnesium carbonate etc. Treatment may affect solubility, bioavailability and chemical form of the organic pollutant and of soil macro and micro nutrients. Decrease in soil ph decreases the availability of nitrate and chloride.

2. Temperature: Temperature affects (a) bacterial metabolism (b) growth rates (c) soil matrix and (d) physic chemical state of the contaminants. Generally, in situ bioremediation is carried under mesophilic conditions (20º to 40º C).

3. Water content geological characters: Water content especially water availability influences bioremediation rates. Water in soils or sediments may not be available to microorganisms because it is absorbed to solid substances or tied up as water of hydration to dissolved solutes. This can be solved up by irrigating the contaminated soil, compost, heaps and biopiles.

4. Nutrient availability: Nutrients are generally supplemented in both in situ and ex situ bioremediation of soils, ground and surface waters. Nutrient requirement depends on the nature of contaminants and the extent to which polluted site has been subjected to agricultural use.

5. External electron availability: Oxygen is used as an electron acceptor to increase bioremediation activity. A number of aerobic bacteria can break down a variety of aliphatic and aromatic organic compounds of both natural and anthropogenic origin wholly or partially by denitrifying bacteria by sulphate, iron, and molybdenum reducers and by methanogenic consortia.

6. Bioavailability of pollutants: Anthropogenic organic polymers such as polystyrene PVC etc. Are highly recalcitrant because of their insolubility and the lack of extracellular microbial enzymes capable of catalyzing depolymerisation. However, non-polymer degrading bacteria and actinomycetes are able to degrade oligomeric polystyrene fragments and low molecular weight fragments of lignin resulting from fungal attacks on the lignin polymer.

7. Co-metabolism: Co-metabolism is a process whereby microorganisms involved in the metabolism of a growth promoting substrate also transform other organic contaminanats which can be called as co-substrates which are not growth supporting if these cosubstrates are provided as the only sources of carbon and energy.

8. Gene expression: The ability of indigenous microorganism to degrade organic pollutants is dependent on expression of the genes encoding the required enzymes. These genes may not express if these substances are available in very low concentrations. This can be overcome by adding substances that are structurally related to the organic pollutants which will act as inducers.

9. Bio-augmentation: Where degradative microbes do not exist or where the process is too slow, microbial inoculants may be added to enhance bioremediation rates. This technique is known as bio-augmentation and may involve (a) addition of natural isolates of bacteria or (b) genetically engineered organisms.

8.4 Biodegradation

Biological degradation or biodegradation is generally considered as a phenomenon of biological transformation of organic compounds by living organisms, particular microbes. Decomposition of biodegradable substances may include both biotic and abiotic steps. Biodegradable matter is generally organic material that is a source of nutrients for microorganisms. The role of microorganisms in the decomposition of sewage and other organic wastes is long known. It has been considered as a natural process in the microbial world as carbon and energy source for their growth and takes a pivotal role in the recycling of materials in the natural ecosystem. It brings about changes in the molecular structure of a compound ultimately yielding simpler (mineralization and comparatively harmless (non-toxic) products like CO_2, H_2O, NH_3, CH_4, H_2S, or PO_3. When the compound is not fully broken, it is termed as **biotransformation**.

Many of the recalcitrant substances produced by biotransformation may sometimes be more toxic than the original compound. Such changes are brought about by the catabolic activities

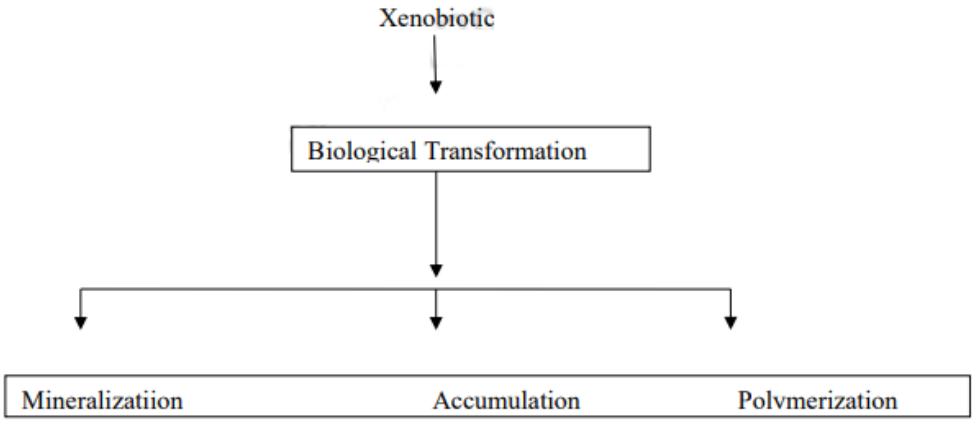


Fig 6: Biological fate of Xenobiotic compounds

of bacteria or fungi by their intracellular or extracellular enzymes secreted in the medium. Biological fate of xenobiotic compounds in the environment can be indicated as shown in fig 6.

Perhaps biosynthetic abilities of the living world and the possibility of the degradation by catabolic enzymes evolved parallel but slowly in nature. This has apparently ensured that

under suitable conditions all natural organics get decomposed and are not deposited in the environment with the exception of natural polymers like lignin and soil humus getting degraded very slowly. This has helped microbes to act as scavengers and reduce the pollution load in natural ecosystem. Bioremediation of polluted environment capitalizes on the activities of aerobic or anaerobic heterotrophic microbes.

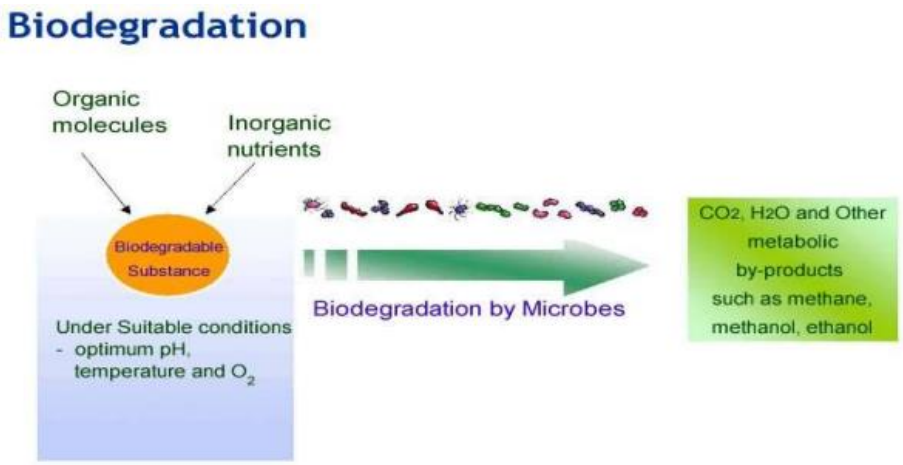


Fig 7: Biodegradation

Many of the wastes are generally complex in nature. While a particular strain of a microbe may degrade only one type of compound or its related group, for some chemical substances the synergistic action of microbial communities or consortia in a polyculture, displaying wide range of degradative abilities rather than a monoculture is desirable.

Sometimes when the degrading material does not serve as a sole source of carbon and energy (non-growth substance) for the organism, but it associated with another growth substrate, then also its gets bio-transformed and the phenomenon is termed as *Pseudomonas putida* in breaking Trichloroethylene.

The factors that affect biodegradation *in situ* are temperature, pH, redox potential, availability of nutrients supply, biomass of the degrader, competition among microbial communities and the nature and concentration of the substrate as well.

The chemical nature of the compound has also great influence in the Process, which is as follows.

(i) Aliphatic compounds are degraded more easily than the aromatic ones. Algae and fungi cannot cleave aromatic rings, whereas bacteria can.

(ii) Recalcitrance of a compound increase with increased branching, polymerization, and presence of polycyclic and heterocyclic residues.

(iii) Water soluble compounds are easier to degrade than insoluble forms.

(iv) Alkenes are easier to degrade than alkanes, while alkanes are more amenale than aromatics.

(v) For aromatics, degradability may be influenced by molecular orientation e.g. ortho > para > meta.

(vi) Halogen, nitrogen and sulfonate substitutions inhibit biodegradability.

Some of the microbes which can degrade various chemicals are:

Chemicals	Microbes
1. Hydrocarbons	*Pseudomonas*, *Nocardia, Arthobacter, Mycobacterium*
2. PCBs	*Pseudomonas, Candida, Alcaligenes*
3. Phenolics	*Pseudomonas, Flavobacterium, Trichosporon, Bacillus*
4. Naphthalene	*Pseudomonas, Nocardia*
5. Organophosphates	*Pseudomonas*
6. Benzene	*Mycobacterium, Alcaligenes*

8.4.1 Aerobic and Anaerobic degradation

Microbial degradation or transformation of organic compounds may involve either of the two processes of aerobic (oxygen dependent) or anaerobic situation, while in some cases it may lead both conditions to detoxify some xenobiotic compounds. (Xenobiotic refer to unnatural, foreign and synthetic chemical such as pesticide, herbicide etc.)

8.4.1.1 Aerobic degradation

In the conventional aerobic system, the substrate is used as a source of carbon and energy. It serves as an electron donor resulting in bacterial growth. The extent of degradation is correlated with the rate of O_2consumption, as also previous acclimation o the organism in the same substrate.

Two enzymes primarily involved in the process are di- and monooxygenases. The latter enzyme can act on both aromatic and aliphatic compounds, while for the former, only

aromatic compounds can act as substrates. Another class of enzymes involved in aerobic condition are peroxidises, which are receiving attention recently for their ability to degrade lignin.

8.4.1.2 Anaerobic degradation

This process is of widespread occurrence and relies on the metabolic versatility of mixed microbial populations present in soils or sediments when O2 supply is limited.

Growth yield of anaerobic bacteria is extremely low due to low energyyields. It has drawn attention these years due to the possibility of decomposition of extremely recalcitrant xenobiotics through this process.

Though the anaerobic process is slow, needs long retention time and produce H2S gas, yet it is more advantageous than the aerobic one due to its non-dependence of O2 supply.

Three temperature ranges are used in anaerobic degradation:

- Cold digestion at about 20ºC
- Mesophylic digestion at about 20º - 40ºC
- Thermophilic digestion at about 40º-55ºC

The anaerobic methods of waste water treatment are considered safe, since few toxic chemicals can be stripped into the ambient air. Chlorinated xenobiotics need to be dehalogenated to make harmless and biological treatment is an attractive proposition.

They mostly need anaerobic situation for dehalogenation by bacterial genera like Pseudomonas, Anthrobacter, Mycobacterium,etc. Unlike aerobic condition, in an anaerobic degradation the chlorinated molecule is used as a direct source of electron.

8.4.2 Sequential Degradation

In many cases, both anaerobic and aerobic sequences are combined. This helps in the reduction of toxicity and mineralization of compounds, which are otherwise recalcitrant. For example, tetrachloroethylene and tetrachloromethane may be mineralized in sequential steps of anaerobic and aerobic conditions, so that initially TCE and chloroform are formed, which later in aerobic methanogenic stage, are converted into CO2 and H2

Herbicides and pesticides Biodegradation

With the advent of Green Revolution, there had been a quantum jump in the use of synthetic herbicides and pesticides throughout the world to sustain high yielding crop varieties. They have now become a part of modern agriculture. Some of the common herbicides based on the chemical group are- Propham (Carbamate), Dicamba (Aromatic acid), Propanil (Anilides), Simazine, Atrazine (Triazines), Glyphosate (Organophosphate). Many of them are highly toxic, persistent and show recalcitrant to natural decomposition.

LD50 values of some pesticides (in rat)

Herbicide	LD50 value (mg/kg body wt)
Simazine	5000
Glyphosate	4050
Dacamba	1700
MCPA	700

A number of them are found to contaminate the surface and ground waters through run off from agricultural fields. Commonly used ones are triazine derivatives, carbamates, organophosphates, aldrin, parquat, diuran, parathion, malathion, etc. Some have even been found to be carcinogenic. Herbicides could be partly decomposed in soils through chemical or photochemical reactions. However, the biodegradability is very much variable. Some of the pesticides may appear as recalcitrant, but can be degraded by the process of co-metabolism. A number of microbial cell bound enzymes and extracellular ones can catalyze the breakage of bonds in herbicide molecules in soil and thus, help in the detoxification of some of them.

Hydrocarbons Biodegradation

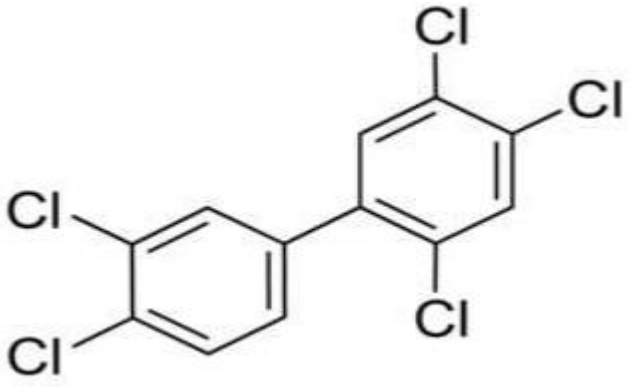
The ability of microbes to degrade hydrocarbons was first pointed out by Zobell (1950). Interest in this process is particularly implicated with the abatement of oil pollutions and oil spills, over and above its utility in the release of oil from oil bearing substrates (prospecting). Many other bacteria, yeasts and filamentous fungi have also shown this capacity. They include species of *Pseudomonas*, *Mycobacterium*, *Corynebacterium*, *Nocardia*, *Arhrobacter* and *Candida*.

Biodegradation of some specific wastes

a) Polycyclic aromatic hydrocarbons (PAHs): These include naphthalene, phenanthrene, anthracene. They are hazardous compounds originating from oil tar, wood preserving creosote and fossil fuel combustion. They are degraded very slowly.

Biotechnological method for remediation of PAH applies *Pseudomonas spp*. and when incubated at 30ºC., gave optimum degradation result. This helped develop strategy for bioremediation of PAH-polluted soils and waste streams.

b) Polychlorinated biphenyls (PCBs): They are halogenated aromatics with the empirical formula $C_{12}H_{1-n}Cl_n$, where n = 1-10. Structurally they have aromatic biphenyl ring substituted with chlorine, viz. Pentacholorobiphenyl. They are a class of compounds produced either commercially or are found as byproducts of combustions. They are used in electrically conductivity as pesticides, plasticizers, microscopic oils, adhesives, paints etc. The industrial value is due to thermal and chemical stability, general inertness and resistance to corrosion. Use of PCBs is now dramatically reduced and mostly restricted to transformers. They accumulate in soil and sediments due to hydrophobic nature.



c) Organophosphates: These are classed as a group of pesticides, but also have some industrial applications as oil additives and plasticizers. Unlike organochlorides, they do not generally bioaccumulate, but undergo hydrolysis and biodegradation under suitable conditions. Detoxification can occur through conjugation with cellular glutathione (GSH).

8.5 Food, Feed and Energy From Solid Waste

8.5.1 Basic Concept of Energy

Energy is an essential part of our daily life. Electric current has been used for many purposes. Such as functioning several appliances, heating, running machinery and for creating radiowaves that carry information over thousands of kilometres, lighting etc. Electricity is also generated by releasing water from a height for running a turbine. Energy such as heat, light, electric and mechanical which are different forms of energy are interconvertible. All life processes are also based on energy. The food energy sustains

biological functions of organisms. Humans and animals muscle energy is derived from food for doing work through its conversion as heat energy by releasing the chemical energy through oxidation stored in the mechanical bonds of food materials. The primary source of all forms of energy in the earth is the solar energy and because of this life sustains on the planet. This energy gets converted to chemical energy in the process of photosynthesis in which water and carbon dioxide gets converted into carbohydrates and other organic compounds. In definite situations these compounds are changed into coal, oil and natural gas through geological processes and when burnt, give heat energy by releasing chemical energy. It is important to note that the amount of solar energy that falls on .the planet's surface each year is equal to the energy supplied by 227 million tonnes of coal, indicating about one billionth of its total output. The rest streams out in all directions into space.

8.5.2 Energy Generation from Waste

The largest source of energy are municipal wastes and for any large-scale plant it will be the main fuel. It is composed of several fractions of which domestic refuse only comprises between 50% and 70%. Other wastes including general industrial wastes which is very similar to immense municipal waste, fragmentizer waste from car shredding, wood waste, hospital wastes, straw and chicken litter can all be utilized where they are of large enough quantity as economic outlet for the energy.

The generation of electricity from waste using energy is a 'green' activity for two reasons Firstly, the waste of great majority is part of the natural carbon cycle and its carbon content will return to the atmosphere as CO_2 whether by being broken down by bacteria or by being burnt. Therefore, for displacing any fossil fuel, the waste used, would add extra CO_2 to the atmosphere, and will help to limit the greenhouse effect. Further, the bacteria convert much of carbon to methane. Secondly, as a fuel has had no energy spent on it as opposed to coal, oil or even gas, which will have had a considerable amount of energy spent on them by the time they enter a boiler, such energy will have already made its contribution to the pollution of the atmosphere.

The processing waste provides a prospect to increase the amount of both recovered ferrous and non-ferrous metals and as regards paper and plastic - if indeed the recycling of the latter is economic. These materials are probably only kept out of the waste stream for a couple of

cycles before they enter it for good. The maximum amount of recycling that was likely to take place, it would have little effect on the recovery of energy from waste due to the maximum amount of recycling which was likely to take place.

8.5.3 Magnitude of Problem and need for its solution

The characteristics of various quantities of generated industrial waste are not accurately known in many cases. It may leads to serious environmental degradation because of their unsystematic and hazardous way of management. In order to meet this difficulty, the Government of India has set-up the National Waste Management Council. The purpose of this is to:

- Identify the wastes;
- Suggest technological alternative for:
    - Reduction,
    - Recycling,
    - Reuse, and
    - Research & development
- Determine categories of areas where specific measures will be needed; and
- Formulate action points as a basis for executive orders to be issued by Government including legislation, taxes and incentives.

The only way to tackle industrial wastes is to regard it in terms of a management issue rather than in terms of a problem. This would give the approach to this issue a more realistic and practical sense. Reusable, reprocessable or saleable material which otherwise have at the end of the pipe being discarded as wastes could be viably recovered. For this, a cost-benefit analysis taking into account additional process equipment needs to be made. It is necessary to take a look at the best available technology both in terms of efficiency and environmental compatibility. The more important part is to realize the fact that it is damaging for any industrial enterprise in the long turn to be anything other than environmentally friendly.

Solid Wastes Energy: Energy can neither be created nor be destroyed, it may be changed from one form to another form, while the entire energy in the universe remains constant and

never changes, which is stated by the first law of thermodynamic. Actually, there is energy resource scarcity not energy scarcity. The energy resources are depleting nor the energy. For example, the energy resources like oil, coal, gas, firewood are fast depleting. That is why, energy resources scarcity is reported all over the world and in many parts of India, including rural and urban areas. To overcome the growing shortages of oil, gas, electric power and fuel wood, possibilities of recovering energy from all types of wastes are under serious consideration.

Definition of Solid Wastes

• A wide variety of solid materials, as well as some liquids in containers, which are leftover, unwanted or rejected as being spent, useless, worthless or in excess are called solid waste.

The solid wastes are all the wastes arising from human, animal activities.

• In fact, solid wastes include all solid or semisolid materials that the possessor no longer considers of significant value to retain. The term 'refuse' is also used interchangeably with the term 'solid wastes'.

• Solid wastes come from different areas like residential, commercial, industrial and agricultural areas. The solid waste generated in the residential and commercial areas together are called as 'Municipal solid wastes.'

Characteristics of Solid Wastes: Solid wastes have the following three characteristics viz.,

(i) Density,

(ii) Physical and chemical composition i.e., quality,

(iii) Quantity.

The amount of energy recovery can be affected by the density, weight, physical and chemical compositions of the solid wastes. The geographic location, season of the year and length of time in storage can affect the characteristics of solid wastes. The variations in the characteristics in turn, affect the energy recovery. Moreover, the information about the density, weight, physical and chemical compositions are absolutely necessary for assessing the feasibility of resource and energy recovery, for evaluating alternative equipment needs, system, solid waste management programmes and plans.

Measuring methods for the Density of Solid Wastes: National Environmental Engineering Research Institute has prescribed a standard method to measure the density of solid wastes (NEERI, 1974). According to this method the solid wastes samples should be collected from the refuse mass (from dustbins, trucks and waste disposal sites). Then the samples have to be weighed and emptied into a box of one cubic meter capacity. This procedure should be continued till the box is full. The box should be filled and weighed thrice to get an average value of density of solid wastes.

Determining Physical Composition of Solid Wastes: The solid wastes are heterogeneous in nature. So to determine its physical composition is very difficult. Random sampling techniques are required to determine the physical composition. The refused samples have to be randomly collected from the refuse collecting centers, vehicles and dustbins. To get good results, a large sample size is recommended. The samples can be taken up to 45 kg.

Method of Measuring the Quantity of Solids Wastes and Generation of Solid Waste: The solid wastes quantities can be measured by using volume or weight. It is better to express the solid wastes quantities in terms of weight rather than volume, because, the use of volume as a measure of quantity can be misleading. To estimate the amount of solid wastes generated in an area, the following methods are used:

1. Weight- Volume Analysis: The solid wastes are loaded from different areas and collected at collection centres. Weigh each load that comes to the collection centres and calculate the weight and volume of each load for each area. Then find out the quantity of solid wastes generated in each area. Though it is an accurate method, it requires considerable effort, time and money.

2. Load-Count Analysis: Take the number of loads (Trucks, bullock carts, lorry) and their volumes and find out the weight of some sample (after deducting the weight of the vehicle) loads from one locality or all localities for one day or up to 7 days. Then multiply the number of loads operated in a day and the average weight of the load to get total weight of the solid wastes generated in an area.

3. Materials-Balance Analysis: Take an individual home or a commercial establishment. Find out how much of materials flow into that home or commercial establishment. Find out

how much material or products and solid wastes come out from that home or commercial establishment. Based on such details the solid waste generation rate can be worked out.

8.5.4 Energy Conversion Technologies

Three major types of technologies are available to extract energy from the solid wastes (Table 1).

Table 1. Energy from solid waste: Processes and Fuels	
Types of processes	**Types of fuel**
A. Combustion Process	1. Electricity 2. High Pressure Steam
B. Dry Chemical Process a) Pyrolysis b) Gasification c) Hydrogasification	1. Oil 2. Gas 1. Low- medium Energy gas-Methane 2. Electricity 1. Methane 2. Ethane
C. Aqueous Process a) Chemical Reduction b) Alcoholic Fermentation c) Anaerobic Digestion	 1. Oil 1. Ethanol 1. Methane

Anyone of the methods can be chosen depending upon the resources to be recovered and types of energy to be extracted. A brief note on each process of extracting energy is given below:

8.5.4.1 Combustion Process

Combustion process is direct burning of the solid wastes. By burning the wastes steam is generated which is used to produce power with the help of turbine process. The energy efficiency upto 30% is recorded even under controlled conditions. Therefore, the wastage of combustible matter is higher in this process.

8.5.4.2 Dry Chemical Process

a) Pyrolysis: In this process, the solid wastes can be converted into oil, gas and carbon char. The organic material is destructively distilled in the absence of air or oxygen. The process is shown in a flow sheet (Fig: 1). Shredder helps to reduce the solid wastes into fine particle size.

The ferrous metals are separated with the help of magnet. Air classifier helps further to remove the inorganics. Froth floatation helps to recover glass from the inorganics. Eddy current separator separates aluminum from the inorganics. The second shredder further reduces the refuse into a fine particle size. These fine particle size solid wastes reach the flash pyrolysis reactor. Flash pyrolysis occurs in the reactor. The cyclone separator separates the solid char from the product fluids. For the formation of oil, the temperature is kept at 500°C; and for the formation of gas and char, the temperature is kept at 700°C. The carbon char can be modified into briquettes for use as a solid fuel. Mostly this pyrolysis technique is used for manufacturing liquid fuel from the solid wastes. This process also has certain advantages and disadvantages.

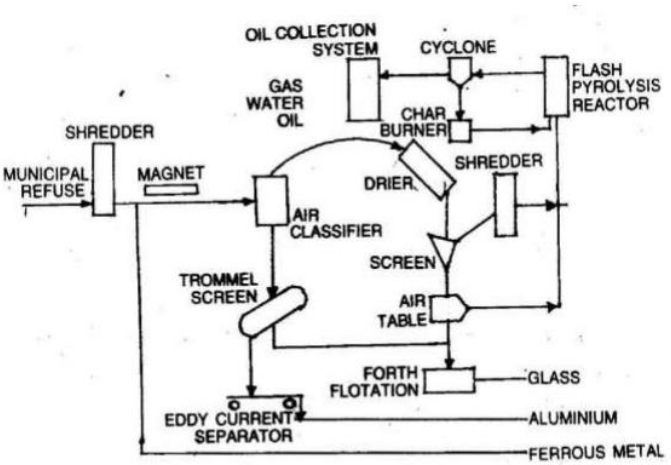


Fig. 1 Pyrolysis Process

b) Gasification: In this process (known as US Purox Process), the solid wastes are heated in a reactor in the presence of limited quantities of oxygen to get synthesis gas consisting of carbon monoxide and hydrogen. The process is shown in a flowsheet (Figure 2). The oxygen which comes from the cryogenic air separation unit helps to maintain the temperature at 1600-1700°C in the partial combustion zone. In this zone at that temperature, the non-combustible substances are fused, whereas the organic materials are degraded to gases, liquids and solid char. As the gas rise and get dried, they preheat the charge

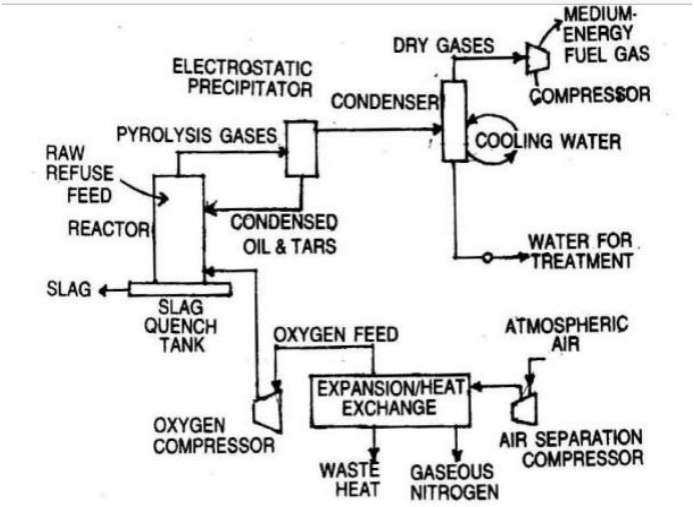


Fig. 2 Purox Process

before exiting at 250°C. The electrostatic precipitator helps to remove the condensed oil droplets, tars and fly ash. They are recycled to the reactor for degradation to gases. The hydrogen sulphide and organic acids are separated from the gases by a neutralizing solution present within an acid absorber. In a condenser, the moisture is removed from the saturated gas. This way the gas is obtained from the solid wastes. But the gas obtained is not having equal heat value as that of natural gas.

The gas produced by Purox process can be made equal to natural gas in terms of heat value. To produce the substitute natural gas, the following arrangements are necessary: fixed bed or fluidized bed methanation reactors containing nickel or other suitable catalysts, pelleted catalysts in packed tubes, and pelleted catalyst suspended in a fluid.

The low-medium energy fuel gas produced in the Purox process can be used to produce electricity. To produce electricity, a combined gas-turbine system cycle method can be used. At a gas turbine inlet temperature of 980°C an overall heat rate for the original bio mass raw material of 12 mJ/kWhr could be envisaged, giving a net delivered electricity efficiency of 29.4% (equal to electricity derived from coal).

8.5.4.3 Aqueous Processes

The important three aqueous processes are: (a) Anaerobic digestion, (b) Alcoholic fermentation, and (c) Chemical reduction. To extract energy from the solid wastes, the anaerobic digestion process is suitable. Therefore the anaerobic digestion process is discussed below:

a) Anaerobic Digestion: The solid wastes are converted into methane gas in the anaerobic digestion process. The process of making methane gas is shown in a flow sheet (Fig. 3). The shredder helps to reduce the size of solid wastes into 7.15-15. cm. Magnetic separator and screen help to remove ferrous metal and fine grit and glass. Separation of inorganic and non-digestible material can be achieved by either a dry or wet system. For separation, in the dry system, the air classifier is used, whereas in the wet system a hydropulper is used. Before reaching the digestor, the solid wastes are mixed with nutrients, lime and ferrous salts in the mixing tank. The digestor is a cylindrical tank. Just like biogas unit, in this process also a stirring mechanism is provided along with floating cover. The gas produced in the digester contains methane and the carbon dioxide gas in 50:50 ratio.

b) Anaerobic Digestion of Vegetable Market Waste: All vegetable wastes may be biodegraded anaerobically to produce a composite fertiliser and biogas with suitable starter such as cow dung. However, the conventional biomass digestors cannot be used as they require 10% slurry of waste material. Therefore, it is not economical as it involves large investment. Bio-gas may be sucked out of an open vegetable waste pit having depths in the order of 10-20m using blowers. It may not be possible to suck out the entire gas produced. A considerable portion of gas produced escapes to the atmosphere.

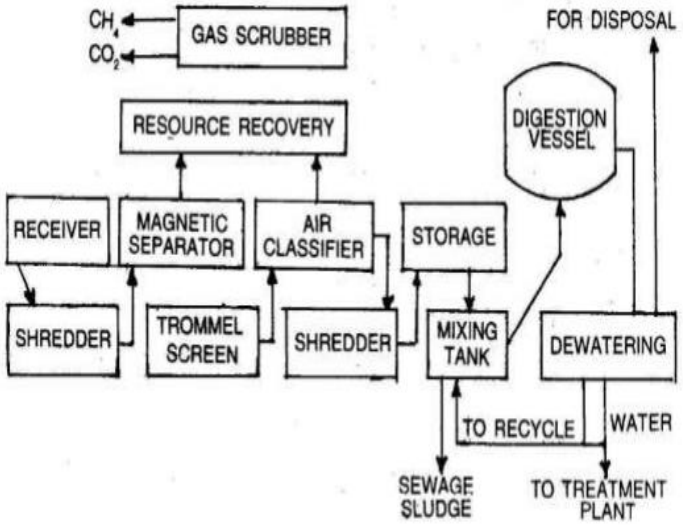


Fig. 3 Anaerobic digestion process

8.6 Health aspects, Toxicology and Allergenicity

8.6.1 Changing Environment and Health

The living organisms can be sustained by the surroundings may also be a insightful basis of ailing health. Rising industrialization, mounting technology and economics, tied with emergent population of world recently have thoroughly changed, as well as still altering, our environment. It has shown a profound impacts on the health and well-being of organisms. Few undesirable effects have been experienced presently involving rising air pollution, acid rain, global warming, and destruction of the ozone layer by CFCs, mounting waste, and others. For the most part serious problems faced today are the liberation of huge amount of array for chemicals within the surroundings. It might causes air, water, soil pollution, as well as blemishing of food. Somewhat, chemicals may be transferred to remote areas where they can draw out unfavourable effects to living organisms.

Environmental pollutions considered as a foremost unease. An increased consciousness pertaining to the effects of air and water pollution and removal of toxic wastes on human health.

"Minamata disease" is a disease arisen from water pollution attracting more attention .The disease have been caused by fish and shellfish laden containing extremely lethal methyl mercury, whether the itai-itai-byo is primarily accredited to using up of rice tainted with elevated levels of cadmium (Cd).

Changes in Disease mould: Alteration in the environment have been associated with the pattern that have been changing and allocation of diseases as well as its effects of health. A analogous mould is obvious in several other countries, involving developing world.

Table 1: Changing causes of Death between 1990 and 1995

Year	1900		1950			1995		
Rank		%	Rank		%	Rank		%
1	Pneumonia-influenza bronchitis	14.4	1	Heart disease	36.6	1	Heart disease	27.4
2	Tuberculosis	11.3	2	Malignant neoplasm	4.9	2	Malignant neoplasm	25.8
3	Diarrhea & enteritis	8.1	3	Cerebrovascular diseases	10.6	3	Unintentional injuries	6.0
4	Heart disease	8.0	4	Accidents	6.8	4	Cerebrovascular diseases	5.3
5	Bright's disease (chronic nephritis)	4.7	5	Pneumonia & influenza	3.1	5	Chronic obstructive pulmonary disease	4.1
6	Accidents	4.5	6	Diabetes mellitus	1.7	6	HIV infection	3.1
7	Congestion & brain hemorrhage	4.2	7	Suicide	1.3	7	Pneumonia & influenza	2.6
8	Disease of early infancy	4.2	8	Chronic liver disease & cirrhosis	1.0	8	Suicide	2.2
9	Cancer & other malignant tumors	3.7	9	Homicide & legal intervention	0.6	9	Homicide & legal intervention	1.8
10	Diphtheria	2.3	10	Chronic obstructive pulmonary disease	.05	10	Chronic liver disease & cirrhosis	1.5

Living organisms together with humans have been exaggerated by pollution. Numerous diseases in humans are perceptible for some compounds in air, water, and foods which have been consumed by human beings. Various agents of industries unconfined within the environment have been considered as carcinogenic (cancer-causing).

Some serious examples related to environmental diseases are: cancer, respiratory diseases, birth defects, heavy-metal poisoning, and injury to the reproductive system. These are briefly examined below:

Cancer: The tangible quantity of deaths commencing from cancer, though, is still expanding.

Even though cancer have been found in children's which is frequently a fatal disease in childhood. About 10% of children's suffering from cancer in childhood are killed.

Birth Defects: The life span of humans contributing the period of maximum vulnerability for exogenous injury is in the premature stages of morphogenesis. Various chemical agents are measured as teratogenic, which causes birth defects. Those several chemical agents can be organic solvents, pesticides, dioxins, and heavy metals like Pb, Cd, Hg, etc.

Reproductive Damage: Recently some studies concluded that various toxic substances might persuade harmful impacts on reproductive systems in animals as well as in humans. Some of the examples are organochlorines like PCBs, dioxins, also DDT; pesticides such as carbamates (e.g., aldicarb, carbofuran), triazines (e.g., atrazine and simazine [herbicides]), and pyrethroids, heavy metals like Cd, Pb, and Hg; organobrominate compounds, and others.

Respiratory Diseases: Numerous studies of epidemiological and animals have revealed that pollutants in the air are mostly originate in the urban environment at high concentrations which have adverse effect on the lungs.

Heavy Metal-Induced Diseases: The construction of heavy metals, like copper (Cu), lead (Pb), and zincs (Zn), have increased significantly. An additional noxious element is arsenic (As). Various metals and nonmetallic elements accrue upto extreme levels due to industrial pollution. Even though chronic poisoning of lead Pb have overwhelmed humans. In recent decades, the significance of Pb as a pollutant of environment have received widely attention. The nervous system, the hematopoietic system and the kidneys have the major prominent adverse effects of Pb,

8.6.2 Toxicology

Toxic substance or toxicant is a substance which is injurious for living organism due to its harmful effects biological system and processes. it is also known as "Science of poisons". This is based upon, if a substance is noxious depending on the kind of exposed organism, the quantity of substance, and the route of exposure. Subjects which are exposed to toxicants in the environment might be in various different physical forms. The substance associated with the toxicant is known as matrix.

A strong effect may be exerted by the matrix upon the toxicity of the toxicant. Numerous variables are available relative to some of the ways within which organisms have been exposed to hazardous compounds.

The concentration of the toxicant is other important factor, that might range from the pure substance (100%) down to a very dilute solution of a highly potent poison. The time of exposure per exposure incident as well as the frequency of exposure are essential.

The site of exposure as well as the route may also affect toxicity. The analogous body parts might be exaggerated by chronic local exposure, for which the time period may be as long as several years.

Systemic chronic exposure differs in that the exposure occurs over a prolonged time period. In case of exposure sites for toxicants it may be essential to consider the foremost routes and sites of exposure, distribution, and elimination of toxicants in the body as shown in Figure 1.

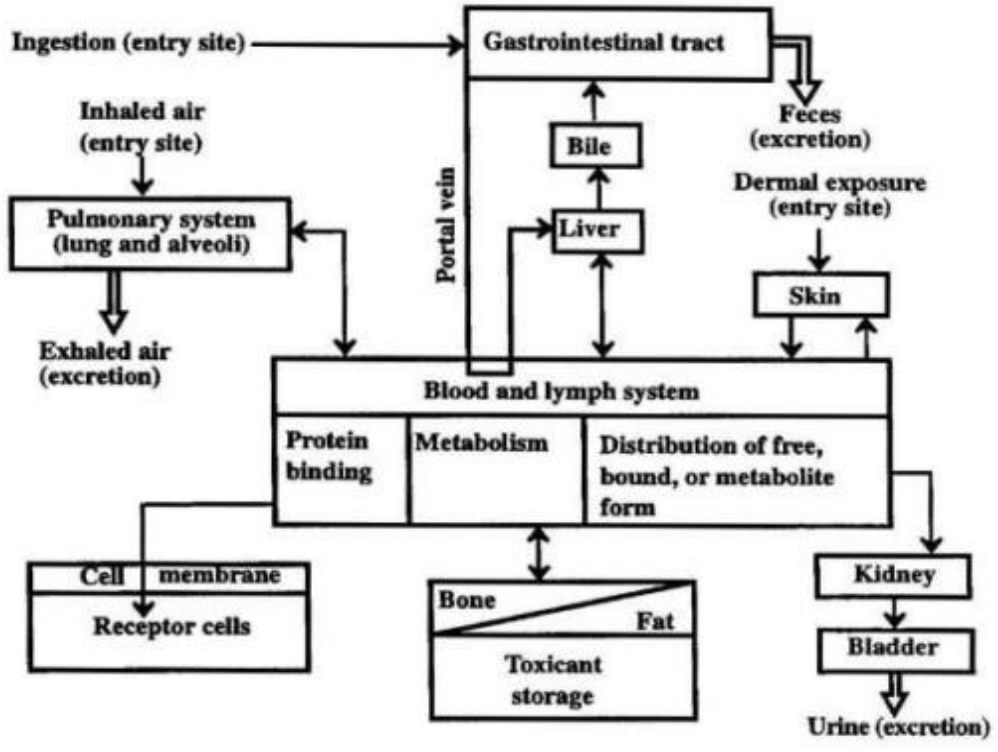


Fig 1: Different routes of exposure, metabolism, storage, routes of distribution and removal of toxic compounds in the body

8.6.2.1 Comparative Toxicities

Table 2 explains the standard toxicity ratings which have been utilized in describing approximate toxicities of several compounds to human beings. Though, as much as a quart of a somewhat toxic compounds may be requisite in order to kill an adult human. While there is a considerable disparity between LD50 values of two distinct compounds, which is having the lower value is called to be more compelling.

Substance	Approximate LD_{50}	Toxicity rating
DEHP[2]	10^5	1. Practically nontoxic, $> 1.5 \times 10^4$ mg/kg
Ethanol	10^4	2. Slightly toxic, 5×10^3 to 1.5×10^4 mg/kg
Sodium chloride		
Malathion	10^3	3. Moderately toxic, 500 to 5000 mg/kg
Chlordane		
Heptachlor	10^2	4. Very toxic, 50 to 500 mg/kg
Parathion	10	
TEPP[3]	1	5. Extremely toxic, 5 to 50 mg/kg
Tetrodotoxin[4]	10^{-1}	
	10^{-2}	6. Supertoxic, <5 mg/kg
TCDD[5]	10^{-3}	
	10^{-4}	
Botulinus toxin	10^{-5}	

[1] Doses are in units of mg of toxicant per kg of body mass. Toxicity ratings on the right are given as numbers ranging from 1 (practically nontoxic) through 6 (supertoxic) along with estimated lethal oral doses for humans in mg/kg. Estimated LD_{50} values for substances on the left have been measured in test animals, usually rats, and apply to oral doses.

[2] Bis(2-ethylhexyl)phthalate

[3] Tetraethylpyrophosphate

[4] Toxin from pufferfish

[5] TCDD represents 2,3,7,8,-tetrachlorodibenzodioxin, commonly called "dioxin."

Table 2: Toxicity scale with example substances

8.6.2.2 Xenobiotic and Endogenous Substances

For a living system xenobiotic substances are foreign substances. While those substances which develop naturally in a biological system are known as endogenous. At a particular concentration range the levels of endogenous substances frequently fall for metabolic processes to generate normally. If the Levels of endogenous substance below a normal range, it causes deficiency response or death as well as same response can develop above

the normal range. This type of retort is described in Figure 2. Some of the Examples of endogenous compounds in living organisms involving several hormones, glucose (blood sugar), as well as few essential metal ions, like Ca2+, K+, and Na.

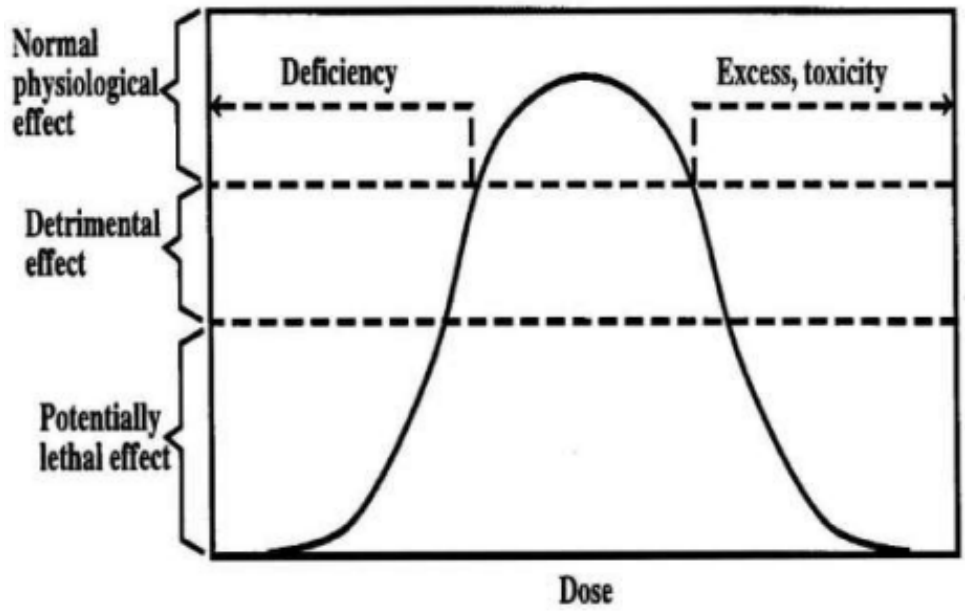


Fig 2: Biological effect of an endogenous compounds in living organisms illustrating optimum level, deficiency and excess

8.6.2.3 Chemistry of Toxicology

Chemistry of toxicology is the branch of science which deals with the chemical behaviour and chemical reactions of toxic substance. Chemistry of toxicology depends up on the chemical properties and molecular structures of molecules and its toxicological effects. It is clearly shown in figure 3.

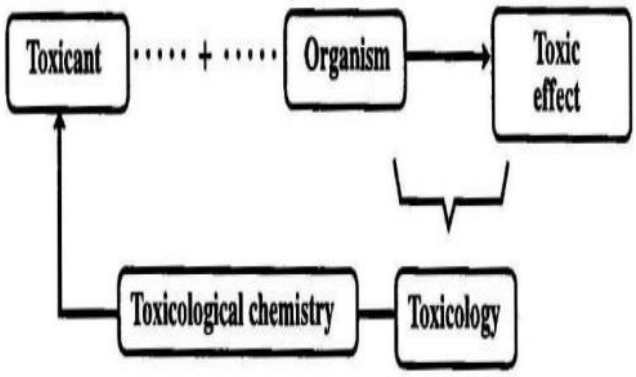


Fig 3: Chemistry of Toxicology

A) Presence of Toxicants in the Body: The processes through which organisms metabolize xenobiotic species are enzyme catalyzed Phase I and Phase II reactions, which are explained concisely here.

Phase I Reactions: In the body Lipophilic xenobiotic species lean to undergo Phase I reactions which makesthem most soluble in water as well as reactive through the fondness of polar functional groups, like -OR group (Figure 4).

Generally Phase I reactions are "microsomal mixed function oxidase" reactions catalyzed by the cytochrome P-450 enzyme system allied with the endoplasmic reticulum of the cell and going on most copiously in the liver of vertebrates.'

Phase II Reactions: Phase II reaction occurs if an endogenous species is fond of by enzyme action to a polar functional group, which results from Phase I reaction on a xenobiotic species. Phase II reactions are also known as conjugation reactions to which enzymes attach conjugating agents to xenobiotics, and its Phase I reaction products, and non-xenobiotic compounds (Figure 5).

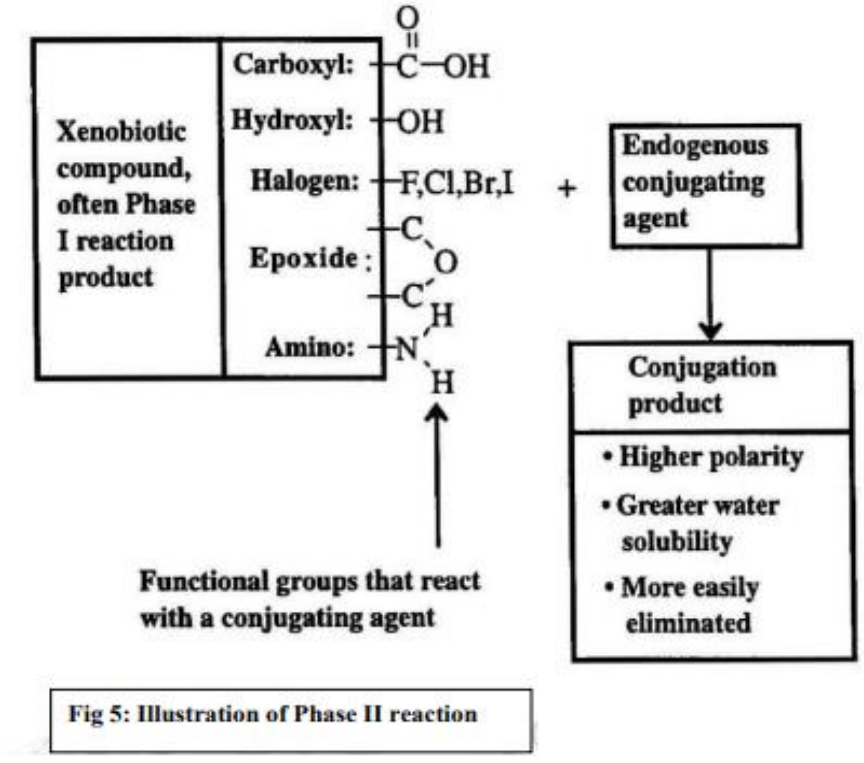


Fig 5: Illustration of Phase II reaction

The conjugation product of this reaction is generally less toxic than the original xenobiotic compound, less lipid-soluble, more water-soluble, as well as more readily removed from the body.

Kinetic Phase and Dynamic Phase

Toxicants present in the body have been metabolized, transported, and excreted; which shows unpleasant biochemical effects; and may generate poisoning. These processes can be categorized into two mainly essential phases, a kinetic phase and a dynamic phase. In the former phase, a toxicant as well as its metabolic precursor of a toxic compound might experience or endure biological activities (such as absorption, metabolism, temporary storage, distribution, and excretion) described in Figure 6.

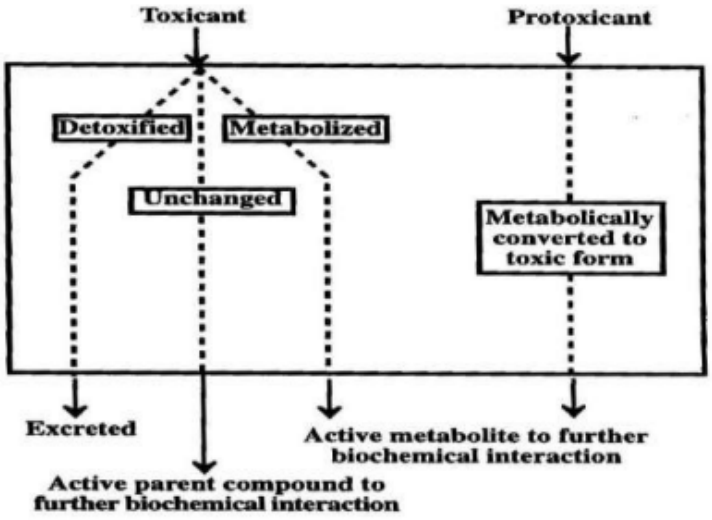


Fig 6: Process involving Toxicants or Protoxicants in the kinetic phase

Absorbed toxicant can be conceded by the former phase unaffectedly as an active parent compound, which can be converted chemically into a detoxified metabolite that have been excreted, or transformed to a noxious active metabolite. When a toxicant (toxic metabolite) merge among the body (cells, tissues or organs) and originate some toxic response is known as dynamic phase (Figure 7)

Three main parts of the dynamic phase:

• Initial reaction with a receptor

• Chemical processes and substances response

• Detectable effects.

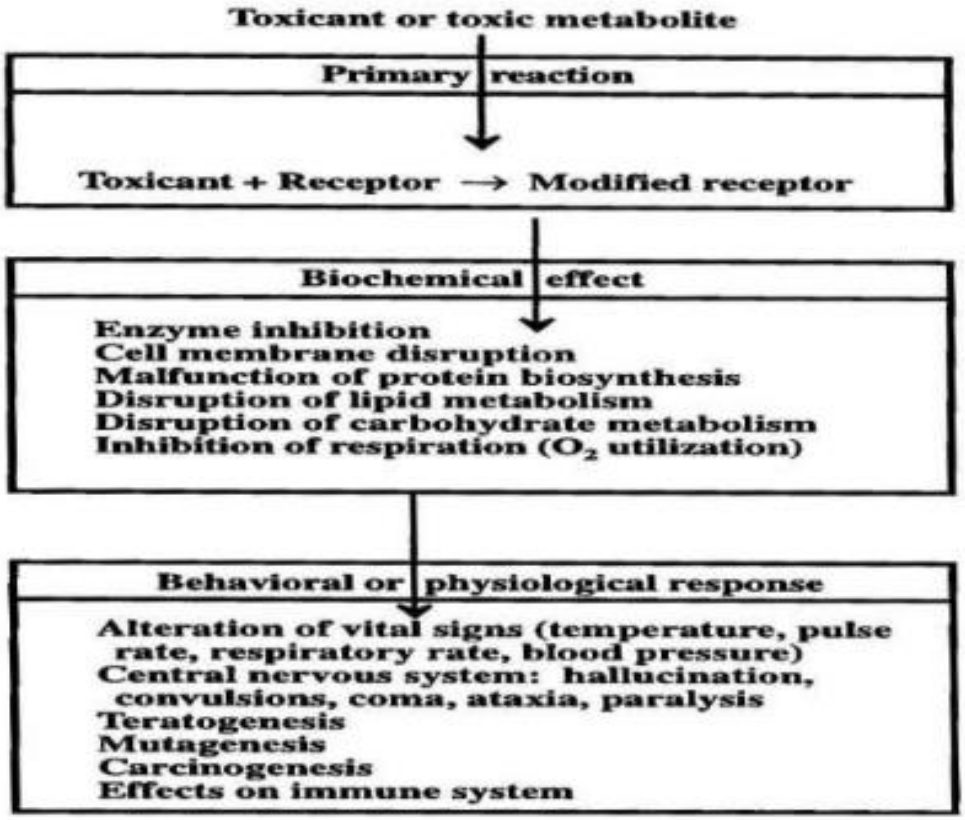


Fig 7: The Dynamic phase of Toxicant action

Immune System Response: Immune system is the natural defence system which protects the body from different types of chemicals; infectious agents and neoplastic cells that ascends to cancerous cells or tissue. Exposure to hazardous substances is an important consequence which gave rise to adverse effects on the immune system of body. Toxic substances may cause immune suppression that easily causes damage in natural defence processes of body. Xenobiotics moreover harm the immune system. It effects on capability of immune system of controlling cell propagation, which results in leukemia or lymphoma.

Estrogenic Substances: The amount of xenobiotic substances has been considered to create undesirable effects on animal as well as reproductive systems of living organisms by being intrusive through the action of estrogens. Experiments of rodents specify that these substances are sometimes known as exogenous estrogens or exoestrogens, might produce diseases of the reproductive tract or system as well as effects like abridged sperm counts and creation of semen.

Hazardous effects on Health: Recently, a wide attention towards toxicology have shifted away from voluntarily predictable, generally harsh, which have created on a limited time scale respectively of concise and extreme disclosure to toxicants, toward deferred, unremitting, due to long duration exposure below the levels of toxicants. Though complete pressure of concluding types of health effects can be extensive. Their appraisal is highly challengeable due to some circumstances.

Health Effects Risks- Estimation: The crucial part in order to estimate the risks of undesirable effects of health as of exposure to toxicants includes extrapolation from experimental data which is observable. The available data is mostly occupied from animals. In a short duration of time it has been exposed to high levels of the substance. For estimation of risk to human population's extrapolation have been done by using linear or curvilinear projections.

Risk Assessment: This is the better ways to what the toxicology is being connected with the region of toxic wastes is in health risk assessment, that provides supervision for managing risk, cleanup, or directive required for hazardous waste depends on knowledge concerning the site as well as the chemical and toxicological properties of wastes. Assessment of risk involves the factors of site characteristics; presence of compounds, involving indicator species; potential receptors; potential exposure pathways; and uncertainty analysis. This can be categorized as follows:

• Hazard identification

• Dose-response assessment

• Exposure assessment

• Risk characterization.

1. Hazard identification: It is the process of determining whether or not a particular chemical is casually linked to particular health effects, such as cancer or birth defects. Because human data are so often difficult to obtain, this step usually focuses on whether a chemical is toxic in animals or other test organisms.

2. Dose response assessment: It is the process of characterizing the relationship between the dose of an agent administered or received and the incidence of an adverse health effect.

Many different dose response relationship are possible for any given agent depending on such conditions as whether the response is carcinogenic or non carcinogenic and whether the experiment is a one time acute test or a long term chronic test. Because most tests are performed with high doses, the dose response assessment must include a consideration for the proper method of extrapolating data to low exposure rates that humans are likely experienced. Part of the assessment must also include a method of extrapolating animal data to humans.

3. Exposure assessment: It involves determining the size and nature of the population that has been exposed to the toxicants under consideration and the length of time and toxicants concentration to which they have been exposed.

Consideration must be given to such factor as the age and health of the exposed population, smoking history, the likelihood that members of the population might be pregnant, and whether or not synergistic affects might occur due to exposure to multiple toxicants.

4. Risk characterization: It is the integration of the foregoing three steps, which results in an estimate of the magnitude of the public health problem.

References:

1. A.K.Chatterji, Introduction to Environmental Biotechnology.

2. G.Bali, R. Rallapalli, S.B.Sullia, A.Shiralipour,S.Kastury, Environmental biotechnology.

3. D.W.Connell, Basic concepts of Environmental Chemistry.

4. C.Baird, Environmental Chemistry.

5. P.Narayan, Environmental Pollution, principles , analysis and control

Unit 9 Principles and concepts

Unit Structure

9.0 Learning Objectives

After studying this unit you will be able to understand:

- the chromosomes and their types.
- properties, types and functions of genes
- sources, types and properties of plasmids and vectors.
- procedure of transfer of genes.
- the enzymes involved in transfer of genes
- How gene transfer is beneficial to humankind?

9.1 Introduction

As you know that cell was discovered by Robert Hooke in 1665. Cell has various cytoplasmic organelles such as mitochondria, endoplasmic reticulum, Golgi body, ribosome, lysosomes, nucleus etc. In these organelles nucleus is one of the most important and play vital role in heredity, it contains genes which are responsible for various characters in organisms. Nucleus was discovered by Robert Brown in the year 1831. Nucleus contains chromosomes, DNA, nucleolus, genes, nucleoplasm, nuclear pores, genes etc. Biotechnology is branch of science in which we studied about the gene transfer from donor cell to recipient cell. Biotechnology provides several useful products by using useful genes microbes, plant, animals. Recombinant DNA technology has made it possible to engineer microbes, plants and animals such that they have novel capabilities. GMO have been created by using methods other than natural methods to transfer one or more genes from one organism to another, generally using techniques such as recombinant DNA technology. GM plants have been useful in increasing crop yields, reduce postharvest losses and make crops more tolerant of environmental stressors. There are several GM crop plants with improved nutritional value of foods and reduced the reliance on chemical pesticides. For attaining GMOs we should have chromosomes, genes, plasmids, DNA fragments etc. Therefore, it is very important to know about the tools which are used in biotechnology. In this unit you will learn about the basic principles & concepts of biotechnology such as chromosomes, plasmids, genes, transfer of genes and usefulness of transfer of genes for human kind.

9.2 Chromosomes

In plant cells chromosomes were first discovered by **Karl Wilhelm Von Nageli** in 1842 and in animal cells chromosomes independently discovered by **Edouard Van Benden**. Their behavior in animal was described by **Walther Flemming** in the year 1882. The name chromosome was invented by **Heinrich Von Waldeyer**.

As you know chromosomes are present in the nucleus of the cell. These are thread like structures. The numbers of chromosomes are fixed in a species for example human being has 23 pairs chromosomes, rat has 21 pairs chromosomes, cat has 19 pairs chromosomes. The nucleus of each cell in our bodies contains approximately 1.8 metres of DNA in total,

although each strand is less than one millionth of a centimetre thick. This DNA is tightly packed into structures called chromosomes, which consist of long chains of DNA and associated proteins. In eukaryotes, DNA molecules are tightly wound around proteins - called histone proteins - which provide structural support and play a role in controlling the activities of the genes. A strand 150 to 200 nucleotides long is wrapped twice around a core of eight histone proteins to form a structure called a **nucleosome**. The histone octamer at the centre of the nucleosome is formed from two units each of histones H2A, H2B, H3, and H4. The chains of histones are coiled in turn to form a solenoid, which is stabilised by the histone H1. Further coiling of the solenoids forms the structure of the chromosome proper. Each chromosome has a p arm and a q arm. The p arm is the short arm, and the q arm is the long arm. In their replicated form, each chromosome consists of two chromatids.

Most bacteria contain a single, circular chromosome. There are some exceptions such as some bacteria i.e. *Streptomyces* which possess linear chromosomes, and *Vibrio cholerae*, has two circular chromosomes. The chromosome together with ribosomes and proteins associated with gene expression - is located in a region of the cell cytoplasm known as the **nucleoid**.

The genomes of prokaryotes are compact compared with those of eukaryotes, as they lack introns, and the genes tend to be expressed in groups known as **operons**. The circular chromosome of the bacterium *Escherichia coli* consists of a DNA molecule approximately 4.6 million nucleotides.

In addition to the main chromosome, bacteria are also possesses extra-chromosomal genetic elements called **plasmid**.

9.2.1 Structure of Chromosomes

The structure of chromosomes: A chromosome is an organized structure of **DNA** and **protein** that is found in cells. A chromosome is a single piece of coiled **DNA** containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. The word ***chromosome*** derived from the two Greek words namely: **chroma** which means **color** and **soma** which means **body**. This name is due to their property of being very strongly stained by particular dyes. Chromosomes vary widely between different organisms.

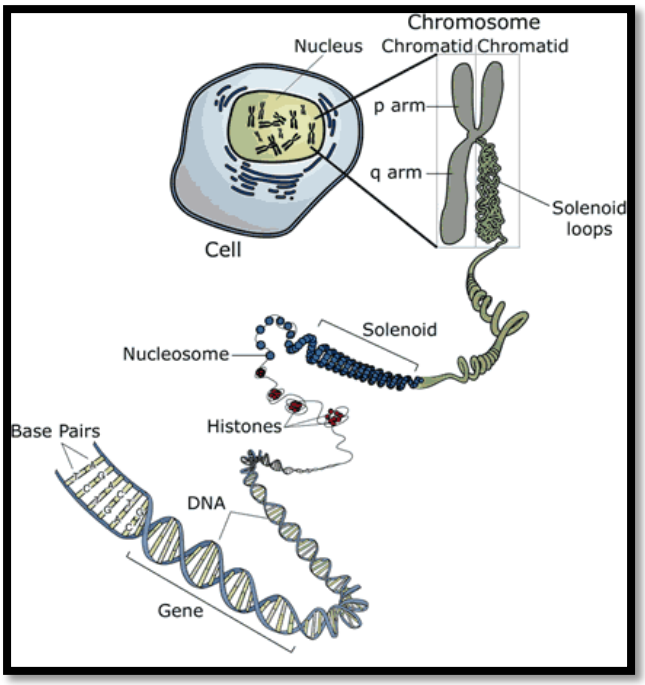


Fig-1: Showing position of DNA, Chromosomes, and genes within cell.

Today we know that a chromosome contains a single molecule of DNA along with several kinds of proteins. A molecule of DNA, in turn, consists of thousands and thousands of subunits, known as nucleotides. A single molecule of DNA within a chromosome may be as long as 8.5cm. To fit within a chromosome, the DNA molecule has to be twisted and folded into a very complex shape. Each chromosome has a constriction point called the **centromere**, which divides the chromosome into two sections, or **"arms"**. The short arm of the chromosome is labeled the **"p arm"**. The long arm of the chromosome is labeled the **"q arm".** The location of the **centromere** on each chromosome gives the chromosome its characteristic shape, and can be used to help describe the location of specific genes. Chemically, the eukaryotic chromosomes are composed of DNA, RNA, histone & non-histone proteins and certain metallic ions. The histone proteins have basic properties and have significant role in controlling or regulating the functions of chromosomal DNA. The non-histone proteins are mostly acidic and have been considered more important than histones as regulatory molecules. Some non-histone proteins also have enzymatic activities. The most important enzymatic proteins of chromosomes are phosphoproteins, DNA polymerase,

RNA-polymerase, DPN-pyropbosphorylase, and nucleoside triphosphatase. The metal ions as Ca+ and Mg+ are supposed to maintain the oragnization of chromosomes.

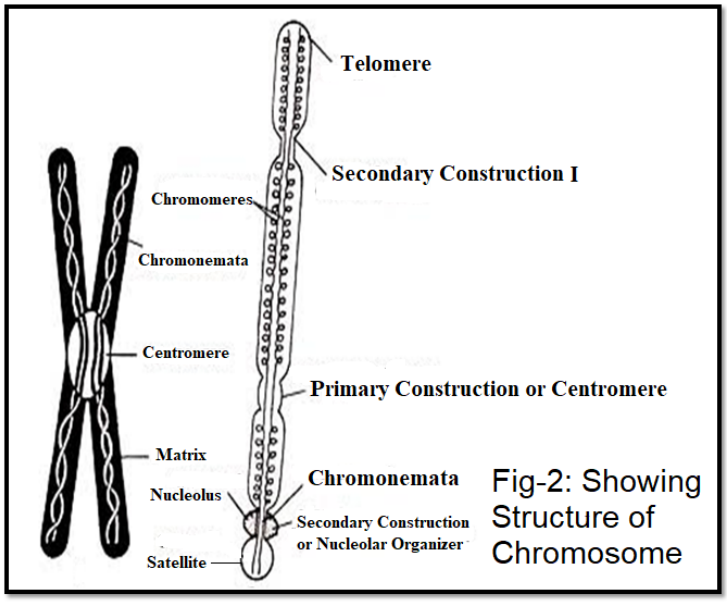


Fig-2: Showing Structure of Chromosome

Molecular Structure of Chromosomes: According to unistranded theory which was illustrated by **Dupraw (1965, 1970)** and **Hans Ris (1967)** each eukaryotic chromosome is composed of a single, greatly elongated and highly folded nucleoprotein fibre of 100Å thick. This nucleo- protein fibre in its turn is composed of a single, linear, double stranded DNA molecule which remains wrapped in equal amounts of histone and non-histone proteins and variable amounts of different kinds of RNA. **Dupraw** produced a **"folded-fibre Model"** to show the ultra-structure of chromosome.

9.2.2 Types Of Chromosomes

There are different types of chromosomes. On the basis of position of centromere chromosomes may be **Metacentric (**A chromosome in which centromere is located in the middle portion, such chromosomes assume V shape at anaphase), **Sub-Metacentic (**A chromosome in which centromere is located slightly away from the centre point or has sub-median position. Such chromosomes assume J shape at anaphase), **Acrocentric Chromosome (**A chromosome in which centromere is located very near to one end or has subterminal position.Such chromosome assumes J shape or rod shape during anaphase), **Holokinetic Chromosome (**A chromosome with diffused centromere. Centromere does not

occupy a specific position, but is diffuses throughout the body of chromosome. Whole body of such chromosome exhibits centromenc activity, also called holocentric chromosome).

On the basis of number of Centromere these may be **Acentric Chromosome (**A chromosome without centromere. Such chromosome remains as laggard during cell division and is eventually lost), **Monocentric Chromosome (**A chromosome with one centromere. It represents normal type of chromosomes), **Dicentric Chromosome (**A chromosome having two centromeres. Such chromosome makes dicentric bridge at anaphase and produced due to inversion and translocations.

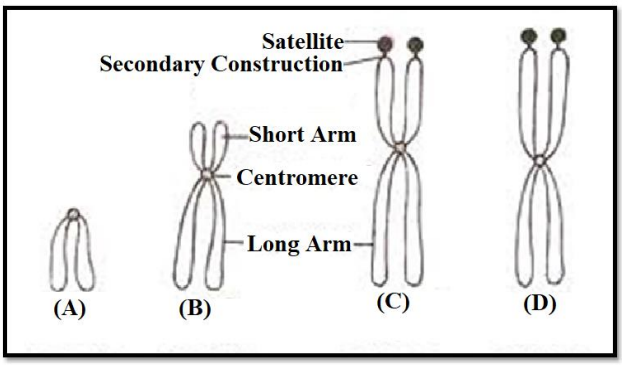


Fig-3: Showing types of chromosomes (A) Telocentric, (B) Acrocentric (C) Submetacentric (D) Metacentric

On the basis of structure and appearance chromosomes may be Linear Chromosome (A chromosome with linear structure or having both the ends free. Such chromosomes are found in eukaryotes), Circular Chromosome (A chromosome with circular shape and structure. They are found in bacteria and viruses).

Special Types of Chromosomes: Chromosomes which significantly differ in structure and function from normal chromosomes are known as special chromosomes. Special chromosomes include lampbrush chromosome, polytene chromosome and B chromosome.

Lampbrush Chromosomes are special types of chromosomes in which large number of loops are projected out from the chromatin axis giving a lampbrush appearance. Such chromosomes are called lampbrush chromosomes. They are found in oocyte nuclei of both vertebrates and invertebrates and spermatocyte nuclei of Drosophila during diplotene stage. Lampbrush chromosomes have remarkable length. They are sometimes larger than polytene chromosomes. The length has been recorded up to 1 mm in Urodela (a order of class amphibia) amphibian.

Polytene or **Giant Chromosomes** first reported by **Balbiani (1881)** in salivary glands of dipteran insects. Later on they were reported in salivary glands of Drosophila and several other insects. Since these chromosomes are generally found in salivary gland, they are also known as salivary gland chromosomes.

A chromosome with two identical arms is known as **isochromosome**. In such chromosomes, both the arms are similar in respect of morphology and gene contents. In other words, both the arms are mirror images of one another. Isochromosomes originate by misdivision of centromere.

9.3 Genes

A gene is basic physical and functional unit of heredity. Genes are responsible for various characters in plants and animals. For example eye's colour, hair type, sex determination and some other characters depend on genes. Genes are typically 103 - 104 base pairs in size although they can be much larger. For example, the human dystrophin gene is 2 x 106 base pairs. *E. coli* has about 4,200 genes which isn't very many considering that at least 1,000 different enzymes are needed to carry out just the basic biochemical reactions in a cell. The smallest genome for a free-living organism (i.e. a cell, not a virus) is that of the bacterium ***Mycoplasma genetalium*** which encodes only 467 genes. Humans are at the other end of the spectrum of complexity and have about **20,000 - 25,000** genes.

9.3.1 Fine Structure of Gene

Benzer (1955) divided the gene into **recon, muton** and **cistron** which are the units of recombination, mutation and function, respectively. Several units of this type exist in a gene. In other words, each gene consists of several units of function, mutation and recombination. The fine structure of gene deals with mapping of individual gene locus. This is parallel to the mapping of chromosomes. In chromosome mapping, various genes are assigned on a chromosome, whereas in case of a gene several alleles are assigned to the same locus. The individual gene maps are prepared with the help of intragenic recombination. Since the frequency of intragenic recombination is extremely low, very large population has to be grown to obtain such rare combination.

Descriptions about Each Genes: There are some genes which are different from normal genes either in terms of their nucleotide sequences or functions. Some examples of such genes are split gene, jumping gene, overlapping gene and pseudo gene.

1. Split Genes: Generally, a gene has a continuous sequence of nucleotides. In other words, there is no interruption in the nucleotide sequence of a gene. Such nucleotide sequence codes for a particular single polypeptide chain. However, it was observed that the sequence of nucleotides was not continuous in case of some genes; the sequences of nucleotides were interrupted by intervening sequences. Such genes with interrupted sequence of nucleotides are referred to as **split genes** or **interrupted genes**. Thus, split genes have two types of sequences, viz., normal sequences and interrupted sequences.

Normal Sequence represents the sequence of nucleotides which are included in the mRNA which is translated from DNA of split gene. These sequences code for a particular polypeptide chain and are known as exons. **Interrupted Sequence** intervening or interrupted sequences of split gene are known as introns. These sequences do not code for any peptide chain. Moreover, interrupted sequences are not included into mRNA which is transcribed from DNA of split genes. The interrupted sequences are removed from the mRNA during processing of the same. In other words, the intervening sequences are discarded in mRNA as they are non-coding sequences. The coding sequences or exons are joined by ligage enzyme.

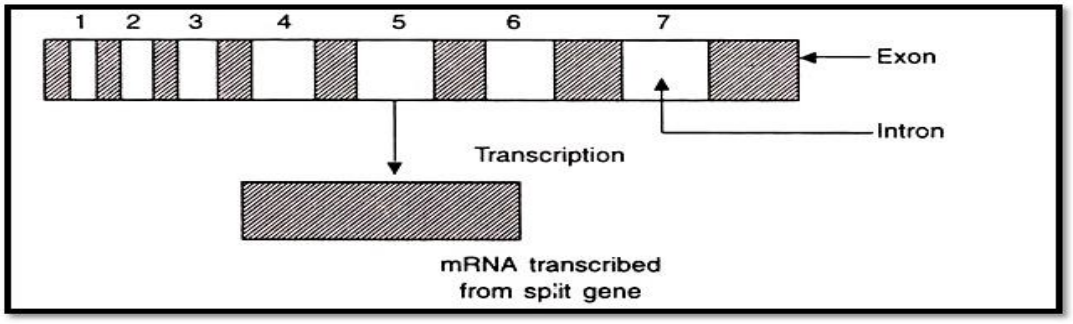


Fig-4: An interrupted gene showing introns and Exons portions

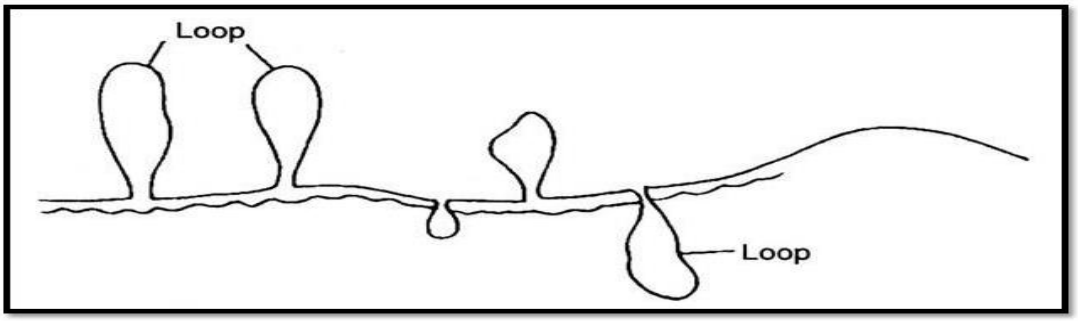


Fig-5: Loop formation in RNA-DNA duplex

2. Jumping Genes: Generally, a gene occupies a specific position on the chromosome called **locus.** However, in some cases a gene keeps on changing its position within the chromosome and also between the chromosomes of the same genome. Such genes are known as **jumping genes** or **transposons**. The first case of jumping gene was reported by **Barbara McClintock** in maize as early as in 1950. Later on transposable elements were reported in the chromosome of *E. coli* and other prokaryotes. In *E. coli*, some DNA segments were found moving from one location to other location. Such DNA segments are detected by their presence at such a position in the nucleotide sequence, where they were not present earlier. The transposable elements are of two types, viz., insertion sequence and transposons.

3. Overlapping genes: Overlapping gene is a gene in which expressible nucleotide sequence partially overlaps with the expressible nucleotide sequence of another gene. Nnucleotide sequence may make a contribution to the function of one or more gene products. It refers to a type of overlap in which all or part of the sequence of one gene is read in an alternate reading frame from another gene at the same locus.

4. Pseudogenes: Gene that resembles a gene but has been mutated into an inactive form over the course of evolution. It often lacks introns and other essential DNA sequences necessary for function. Though genetically similar to the original functional gene, pseudogenes do not result in functional proteins.

9.3.2 Properties and Functions of Genes

There are various properties of genes which are summarized in Fig-6 and also described below:

1. **Forms:** Alternative form of a gene is called as ***allele***. Each gene has two allelic forms. One of these forms in known as **wild type** and the other is known as **mutant type**. Allelic forms are known as dominant and recessive.
2. **Location:** Genes are located on the chromosome in a linear fashion like bead on a string. The location which is occupied by a gene on a chromosome is known as ***locus***.
3. **Status**: Benzer demonstrated that gene consists of several units of cistron, recon and muton which are the units of function, recombination and mutation respectively.
4. **Number:** Each diploid individual has two copies of each gene and gametic cells have one copy of each gene. Each individual has large number of structural and functional features or characters and each character is controlled by one or more genes. Thus, each individual has huge number of genes.
5. **Sequence:** Genes have a specific sequence on the chromosome. The gene sequence is altered by structural chromosomal changes specially translocations and inversions.
6. **Expression:** Gene may be expressed incomplete dominance, complete dominance, over dominance and lack of dominance. When there is lack of dominance, the generation shows characters of the two parents. The gene which is expressed is known as dominant gene and which is suppressed is known as recessive gene.
7. **Change in Form:** The gene may sometimes transform from one allelic form to other. The change in the form of gene is brought out by gene mutation and the changed form of gene is called **mutant gene**.

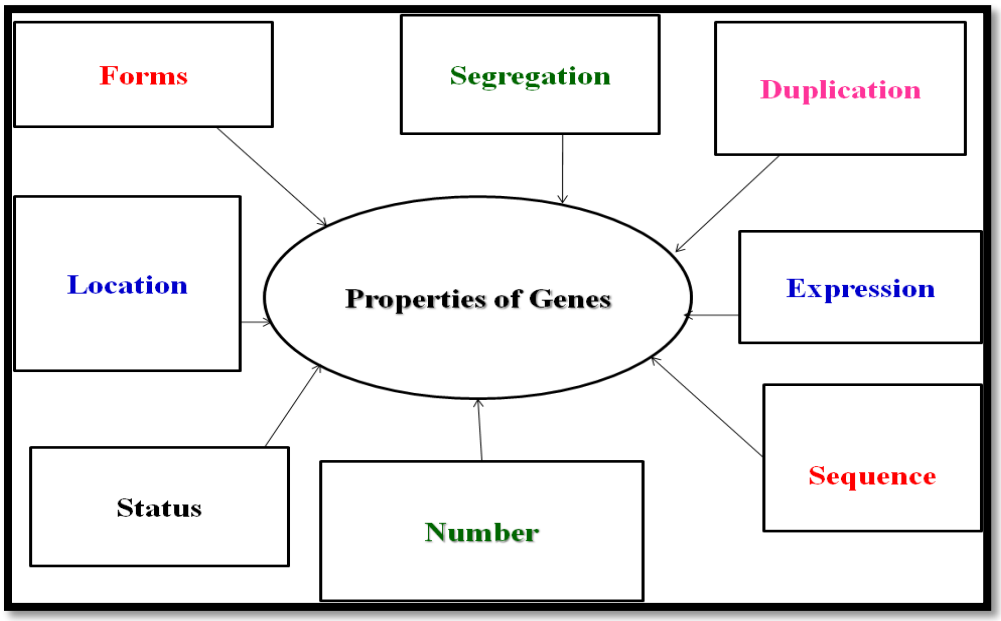


Fig-6 Showing properties of genes

1. **Forms:** Alternative form of a gene is called as ***allele***. Each gene has two allelic forms. One of these forms in known as **wild type** and the other is known as **mutant type**. Allelic forms are known as dominant and recessive.
2. **Location:** Genes are located on the chromosome in a linear fashion like bead on a string. The location which is occupied by a gene on a chromosome is known as ***locus***.
3. **Status**: Benzer demonstrated that gene consists of several units of cistron, recon and muton which are the units of function, recombination and mutation respectively.
4. **Number:** Each diploid individual has two copies of each gene and gametic cells have one copy of each gene. Each individual has large number of structural and functional features or characters and each character is controlled by one or more genes. Thus, each individual has huge number of genes.
5. **Sequence:** Genes have a specific sequence on the chromosome. The gene sequence is altered by structural chromosomal changes specially translocations and inversions.
6. **Expression:** Gene may be expressed incomplete dominance, complete dominance, over dominance and lack of dominance. When there is lack of

dominance, the generation shows characters of the two parents. The gene which is expressed is known as dominant gene and which is suppressed is known as recessive gene.

7. **Change in Form:** The gene may sometimes transform from one allelic form to other. The change in the form of gene is brought out by gene mutation and the changed form of gene is called **mutant gene**.
8. **Exchange of Genes:** The exchange of genes happens between non-sister chromatids of homologous chromosomes due to crossing over and between non-homologous chromosomes due to translocation.
9. **Duplication:** Each gene is duplicated at the time of chromosome duplication. It is believed that chromosome duplication takes place because of gene duplication.
10. **Function:** The primary function of each gene is to control the expression of a specific character in an organism. However, sometimes two or more genes are involved in the expression of some characters. The characters which are governed by one or few genes are called as **oligogenic** traits and those characters which are governed by several genes are called as **polygenic characters**.
11. **Segregation:** Genes in diploid organisms occur in pairs of alleles. The member of a pair segregates precisely like chromosomes during meiosis. Thus genes show segregation during meiosis.
12. **Interaction:** When a character is governed by two or more genes, they sometimes show interaction. In such interaction one gene has masking effect over the other. The masking gene is known as **epistatic gene** and the gene which is masked or suppressed is called **hypostatic gene**.
13. **Linkage:** Sometimes two or more genes are inherited together; such genes are referred to as linked genes. Some genes are linked with a particular sex; they are called as sex linked gene.

Functions of Genes: The concept of gene has been the central point of study from the beginning of 20th century to establish the basis of heredity. The genetic view of gene is based mainly on the **Mendelian inheritance**, chromosomal theory of inheritance and linkage

studies. As you know **Mendel** used the term **factors** for genes and reported that factors were responsible for transmission of characters from parents to their **offspring**. Sutton and Boveri (**1903**) reported that both **chromosomes** and genes segregate and exhibit random assortment, which clearly demonstrated that genes are located on chromosomes. The Sutton-Boveri hypothesis is known as **chromosome theory of inheritance**. Morgan based on linkage studies in **Drosophila** reported that genes are located on the chromosome in a linear fashion. Some genes do not assort independently because of linkage between them. Morgan explained that recombinants are the result of crossing over. The crossing over increases if the distance between two genes is more. The number of linkage group is the same as the number of chromosomes. The chromosome theory and linkage studies reveal that genes are located on the chromosomes. Genetic view is also called as **bead theory**.

The important points about the bead theory are as:

i) The gene is viewed as a fundamental unit of structure, indivisible by crossing over. Crossing over occurs between genes but not within a gene.

ii) The gene is considered as a basic unit of mutation. It changes from one allelic form to another, but there are no smaller components within a gene that can change.

iii) The gene is viewed as a basic unit of function. Parts of a gene, if they exist, cannot function.

Gene Can Function: It was considered earlier that gene is the basic unit of function and parts of gene, if exist, cannot function. **Benzer (1955)** concluded that there are three sub divisions of a gene, viz., recon, muton and cistron. Recons are the regions (units) within a gene between which recombination's can occur. There is a minimum recombination distance within a gene which separates recons. The map of a gene is completely linear sequence of recons. Muton is the smallest element within a gene, which can give rise to a mutant phenotype or mutation. This indicates that part of a gene can mutate or change. This disproved the bead theory according to which the entire gene was to mutate or change. Cistron is the largest element within a gene which is the unit of function. This also knocked down the bead theory according to which entire gene was the unit of function. The name cistron has been derived from the test which is performed to know whether two mutants are within the same cistron on in different cistrons.

Table-1: Showing comparison between chromosomes and genes

Chromosomes	Genes
Similarities	
Each diploid cell has two sets of chromosomes	Each diploid cell has two copies of genes
A gamete (sperm or ova) contains half number of chromosomes than somatic cells.	A gamete (sperm or ova) contains half number of genes than somatic cells.
Each member of homologous pair of chromosomes assorts independently during meiosis (Anaphase-I)	Two or more genes assort independently.
Chromosomes show segregation during meiosis	Genes are also segregate during meiosis.
Chromosomes duplicate during meiosis	Genes also duplicate during meiosis.
Chromosomes mutate	Genes also mutate.
Differences	
Chromosomes are visible under microscope.	Genes are not visible under microscope.
Chromosomes are made up of DNA, histone protein and RNA	Genes are made up of either DNA or RNA

9.3.2 Classification of Genes

On the basis of different aspects genes may be of following types.

On the basis of dominance, genes may be dominant genes and recessive genes. The dominant genes are express in F1 generation while recessive genes whose effect is suppressed in F1 generation.

On the basis of interaction, genes may be epistatic and hypostatic genes. The former that has masking effect on the other gene controlling the same trait. The later, whose expression is masked by another gene governing the same trait.

On the basis of character control, genes may be categorized as major and minor genes. Major gene that governs qualitative trait and such genes have distinct phenotypic effects. Minor gene which is involved in the expression of quantitative trait. Effects of minor genes cannot be easily detected.

1. On the basis of **effect on survival,** genes may be classified as : Lethal genes, semilethal genes, sub-vital genes and vital genes. Lethal gene which lead to death

of its carrier when in homozygous condition. It may be dominant or recessive. Semilethal gene that causes mortality of more than 50% of its carriers. Sublethal gene causes mortality of less than 50% of its carriers. Vital gene does not have lethal effect on its carriers.

2. On the basis of **location,** gene may be of two types: Nuclear genes and Plasma genes. Nuclear genes are found in nuclear genome in the chromosomes. Plasma genes are found in the cytoplasm in mitochondria and chloroplast. Plasma genes are also called cytoplasmic or extranuclear genes.
3. On the basis of **position,** gene are categorized in two types. In this category first is Normal genes and second is Jumping genes. Normal genes have a fixed position on the chromosomes Most of the genes are belong to this category. Jumping genes keep on changing their position on the chromosomes of genome. Such genes have been reported in some species of maize.
4. On the basis of **nucleotide sequence,** genes are of three types: Normal genes, Split genes and Pseudo genes. Normal genes having continuous sequence of nucleotide which code for a single polypeptide chain. Split genes having discontinuous sequence of nucleotide. Such genes have been reported in some eukaryotes. The intervening sequence do not code for amino acid. Pseudo genes having defective nucleotides which are non-functional. These genes are defective copies of some normal genes.
5. On the basis of **linkage,** genes may be three types: Sex linked genes, Sex limited genes and Sex influenced genes. The sex linked genes are located on sex or X-chromosomes on the other hand sex limited genes express in only one gender. Sex influenced genes expression depend on the sex of individual (Example : Gene for baldness in male).
6. On the basis of **Operon model,** genes may be of four types viz. Regulator genes, Operator genes, Promotor genes and Structural genes. Regulator gene found in lac operon of E.coli which directs synthesis of a repressor. Operator gene controls the function of structural genes. Promotor gene initiates mRNA synthesis. Structural gene control the synthesis of protein through mRNA.

7. On the basis of **role in mutation**, genes may be classified as: Mutable genes, Mutator genes and Antimutator genes. Mutable genes exhibit higher mutation rate than other for example white eye gene in Drosophila. Mutator genes enhance the natural mutation rate of other genes in the same genome for example dotted gene in maize. Antimutator genes decrease the frequency of natural mutation of other genes in the same genome. Such genes are found in bacteria and bacteriophages.

9.4. Plasmids as Vector

Plasmid is an extra **chromosomal**, self replicating and double stranded closed and circular **DNA** molecules present in the **bacteria**. It is small, circular pieces of DNA. It **replicates** independently in the **host** cell. Plasmids have revolutionized molecular biology by allowing investigators to obtain many copies of custom DNA molecules. In addition to bacterial chromosome, bacterial cells normally contain genetic elements in their cytoplasm. These genetic elements exist and replicate separately from the chromosome and are called plasmids. The existence of plasmids in bacterial cell was revealed by **Joshua Lederberg** in the year 1952. He was working on **conjugation** process in bacteria. **Lederberg** coined the term **'plasmid'** to refer to the transmissible genetic elements that were transferred from one bacterial cell to another and determined the maleness in bacteria.

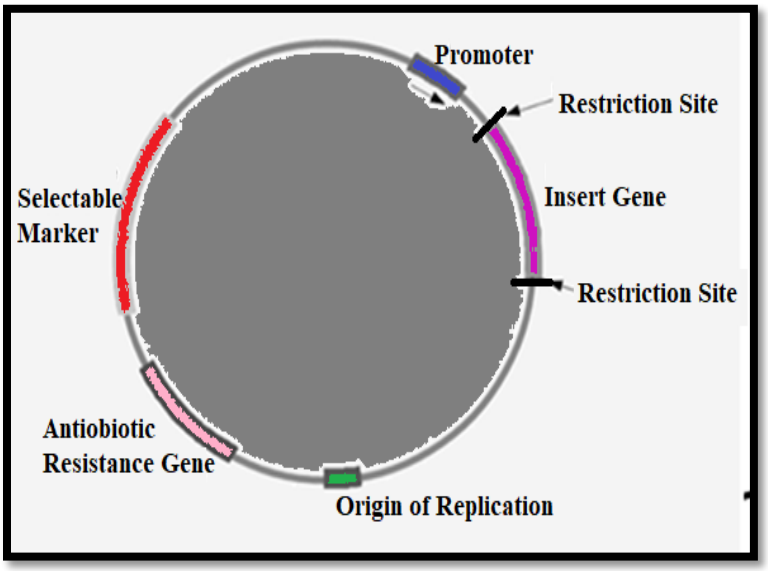


Fig-7: Showing structure of plasmid

9.4.1 Sources of Plasmids

Plasmids are very important tool in biotechnology. As you know plasmids are small, circular DNA molecules that replicate independently of the chromosomes in the microorganisms that harbor them. Plasmids are often referred to as vectors, because they can be used to transfer foreign DNA into a host cell.

Plasmids have been engineered to carry up to 10 kb of foreign DNA and they are easily isolated from microorganisms in the laboratory. Plasmid isolation takes advantage of the unique structural properties of plasmids. Unlike the much larger bacterial chromosome, plasmids are quite resistant to permanent denaturation. Today, most laboratories use commercial kits for plasmid isolations, because the kits are convenient and relatively inexpensive. The kits give good yields of high-quality DNA, while avoiding the need for organic de-naturatants. However, common source of plasmids are bacteria which are found in almost every ecosystems.

9.4.2 Types Of Plasmid/Vector

There are various types of plasmids/vectors have been identified, some of the important types of plasmids are described below:

A. Plasmid Vectors: Plasmid contains genetic information for their replication. They specify a number of host properties. On the basis of these characters they have been grouped into several types.

a) They can be isolated from the cells.

b) They must possess at least one cleavage site for one or more restriction enzymes.

c) Insertion of a linear molecule at one of these sites does not alter its replication properties.

d) They can be introduced into a bacterial cell and cells carrying plasmid with or without insert can be selected or identified.

However, the conjugative plasmids carry transfer genes that enable them to transfer from one bacterium to other. They replicate autonomously but can't transfer to another bacterial cell. In contrast, the relaxed plasmids are found as multiple copies in a cell. Plasmids present typically in 1-2 copies per cell are called **stringent plasmids**. Moreover, the number of

plasmids in a bacterial cell can be increased to about 1000 per cell. This process of increasing the number of plasmids is called **amplification**. Number of plasmids can be amplified by incubation of the host cells with the antibiotic **chloramphenicol**. It inhibits the proteins required for replication of chromosome but does not inhibit plasmid replication. Different species of *Pseudomonas, Streptococcus, Salmonella*, etc. possess a variety of plasmids of different size.

A. **Bacteriophage Vectors:** In simple words, when virus engulfed the bacteria is called bacteriophages. The filamentous bacteriophages are M13, Fd and F1 that contain single stranded circular DNA molecule. They are used for cloning of DNA fragments. In addition, bacteriophage λ is a phage of *E. coli* and contains double stranded DNA molecule of about 49.5 kilo base pairs (kb). The ssDNA (single stranded DNA) exists as a linear molecule that consists of 12 nucleotides long single stranded 5' ends. However, there is possibility to introduce the foreign DNA up to 25 kb in length into the lambda genome without making them incapable to infect *E. coli* and replicate properly. Bacteriophage λ consists of several non-essential regions which can be replaced with almost equal length of foreign DNA molecule to be cloned.

B. **Cosmid Vectors:** Based on the properties of phage DNA and Col El plasmid DNA, a group of Japanese workers showed that the presence of a small segment of phage λ containing cohesive end (cos site) on the plasmid DNA molecule is a sufficient prerequisite for in vitro packaging of this DNA into the infection particles. Therefore, the cosmids are the hybrid vectors derived from plasmids and phage λ (only cos site). Cosmid was constructed for the first time by **Collins and Hohn (1978)**. The special features of cosmids similar to plasmids are the presence of origin of replication, markers, a special cleavage site for the insertion of foreign DNA, and their small size.

C. **Phagemid Cloning Vectors:** Like cosmids, phagemids are also the hybrids of a plasmid and a phage. **Kahn and Helinski (1978)** reconstructed the plasmid of Col El artificially and allowed to get packed in vitro into bacteriophage particles. The phage particles containing plasmid DNA were allowed to infect bacterium. Thus the hybrid vector was termed as phagemids. This insertion of plasmid into phage

genome is reversible and called as lifting' the plasmid. These new genetic re-combinations called as phagemids. The phagemids contains functional ori genes of plagemid and of phage λ.

D. **Shuttle Vector:** The shuttle vectors are the plasmids that are designated to replicate in different host. A shuttle vector is constructed by using bacterial origin of replication in a yeast plasmid. Thus the origins of replication of different host systems such as *E. coli* or yeast are combined in one plasmid. This is why any gene inserted into shuttle vector can be expressed either in bacterium *E. coli* or *Bacillus* or yeast cells. Therefore, shuttle vectors transform E. coli cells with greater efficiency than the original organisms.

E. **F-plasmid:** Fertility factor of *Escherchia coli* K-12 was the first plasmid to be described. **Lederberg et al.** and **Hayes** reported the occurrence of a peculiar infective inheritance mediated by an agent called F which controls system of sex compatibility in *E. coli* K-12 strain. Original K-12 strain and most of its descendants show no mating limitations, but a few derivative strains have more recently been found which are not crossing with one another. These cross incompatible strains are called F-, while strains showing the normal, apparently homothallic condition have been termed as F+.

F. **Col plasmid:** Col plasmids are group of small multicopy colicinogenic plasmids which encode the genes to synthesize colicins. These plasmids need DNA polymerase I for replication and are amplified by chloramphenicol. These plasmids are widely used in gene cloning process. They are also used as a good model for gene replication, transcription and translation.

G. **Suicide plasmids:** These are plasmids which get transferred to another bacterial cell but do not replicate further. These are also known as **mobilizable plasmids**. These plasmids are mostly used for transposon and gene replacement experiment. Most of the suicidal/mobilizable plasmids are based on broad host range plasmids R388 plasmid and RP4 plasmid but there is more advantage if it is based on small narrow host range plasmids.

H. **Virulence plasmids:** Pathogenicity of microbes increases due to these plasmids. Most of them has originated from a single strain and diversified by accumulating traits which is necessary for respective virulence. There are different types of *E. coli* virulence plasmids exist, including those essential for the virulence of enterotoxigenic *E. coli*, entero invasive *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, enteroaggregative *E. coli*, and extra intestinal pathogenic *E. coli*.

9.4.3 Properties of Plasmids

Properties of Plasmids: There are various properties of plasmids which are summarized in Fig. 8 and also pointed below:

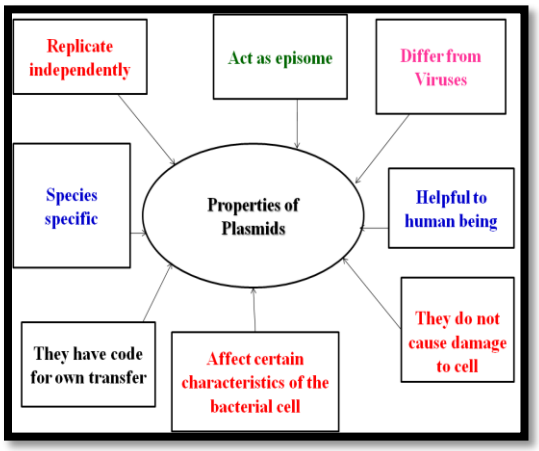


Fig-8: Showing properties of Plasmid

- They are specific to one or a few particular bacteria.
- They replicate independently of the bacterial chromosome.
- They code for their own transfer.
- They act as episomes and reversibly integrate into bacterial chromosome.
- They may pick-up and transfer certain genes of bacterial chromosome,
- They may affect certain characteristics of the bacterial cell,
- Plasmids differ from viruses; they do not cause damage to cells and generally are beneficial.

9.4.4 Plasmids in Ecosystems

Plasmids are found in almost all ecosystems. Extracellular plasmid is found everywhere. Natural plasmid transformation is a means of plasmid acquisition by aquatic bacterial population. Plasmids are released to environment through both abiotic and biotic factors. Bacteriophage infection and predation by protozoa are probably the main biotic factors of

the presence of extracellular DNA in the aquatic environment. The list of naturally occurring plasmids is given in Table-2.

Table-2: Naturally occurring plasmids and the respective traits

Plasmid	Trait	Source
Col	Bacteriocin which kills *E. coli*	*E. coli*
Tol	Degradation of toluene and benzoic acid	*Pseudomonas putida*
Ti	Tumour initiation in plants	*Agrobacterium tumefaciens*
pjp4	2,4-D (dichlorophenoxyacetic acid) degradation	*Alcaligenes eutrophus*
pSym	Nodulation on root of legume plants	*Rhizobium meliloti*
SCP1	Antibiotic methylenomycin biosynthesis	*Streptomyces coelicolor*
RK2	Resistance to ampicillin, tetracycline and kanamycin	*Klebsiella aerogenes*

9.5 Transfer of Genes

Gene transfer describes the introduction of genetic information into a cell from another cell. This process occurs naturally in both bacteria and eukaryotes. Bacteria reproduce by the process of binary fission. In this process, the chromosome in the mother cell is replicated and a copy is allocated to each of the daughter cells. As a consequence, the two daughter cells are genetically identical. In modern days transfer of genes is one of the most important technology in biological sciences. In transfer of genes, desirable genes are transferred from one cell to another. There are various important basic tools which are used in transfer of genes which are described below:

1. Cloning vectors:- Which can be used to deliver the DNA sequences in to receptive bacteria and amplify the desired sequence;

2. Restriction enzymes: Restriction enzymes recognize a specific palindromic sequence of make a staggered cut, which generates sticky ends, or blund cut, which generate blunt ends. Which are used to cleave DNA reproducibly at defined sequences.

3. DNA ligases: Ligation of the vector with the DNA fragments generates a molecule capable of replicating the inserted sequence called recombinant DNA.

You will learn detail procedure of transfer of gene in Unit-11 of this course.

9.5.1 Steps Involved

The important steps of gene transfer are described here:

A. DNA manipulation outside of cells: The key enzymes to manipulating DNA outside of cells are the **restriction endonucleases**. These enzymes cut DNA only at specific nucleotide sequences. Thus, a specific gene may be cut out of a donor cell. Further techniques allow one to specifically change the nucleotide sequence of the isolated gene. Restriction part of the name derives from the actual use of these enzymes by the bacteria that make them: restricting the replication of bacteriophages. The nuclease part of the name means these enzymes **cut DNA**. The *endo* part of the name means that they cut DNA in the middle of double helix strands.

B. DNA transfer to recipient cell (vector):

- To transfer manipulated DNA back into a cell, one typically first inserts the DNA into a plasmid or a vector.
- The vector has to cut by restriction endonucleases
- The desirable gene (DNA) is then inserted into this plasmid.
- Additional enzyme i.e. DNA ligase, then covalently attaches the gene into the vector, thus making gene and vector into one double helix
- The vector may then be transformed into a recipient cell
- Plasmid is allowed to replicate within the host cell.

C. DNA manipulation within the recipient cell

- Once the DNA is in a recipient cell to allow the introduced gene to express, thus changing the phenotype of the recipient cell.
- A second thing that can be done is the gene product can be overly expressed so that the resulting relatively high concentration of protein can be purified and either used for a specific purpose or employed for the characterization of the protein.
- Another thing that can be done is the inserted gene may be sequenced using DNA sequencing techniques.
- The inserted gene may serve as a source of DNA for further cloning of the gene.

D. Making a Recombinant DNA: Recombinant DNA is DNA that has been created artificially.

- Treat DNA from both sources with the same restriction endonuclease.
- The ends of the cut have an overhanging piece of single-stranded DNA.
- These are called "sticky ends" because they are able to base pair with any DNA molecule containing the complementary sticky end.
- Both DNA preparations have complementary sticky ends and thus can pair with each other when mixed.
- DNA ligase covalently links the two into a molecule of recombinant DNA.
- To be useful, the recombinant molecule must be replicated many times to provide material for analysis, sequencing, etc. Producing many identical copies of the same cloning.

E. Cloning

Cloning is a group of replicas of all or part of a DNA. In gene cloning a particular gene is copied or cloned. Cloning often gets referred to in the same breath as genetic engineering, but it is not really the same. In genetic engineering, one or two genes are typically changed from amongst perhaps 100,000. Cloning essentially copies the entire genetic complement of a nucleus or a cell.

Cloning in vivo can be done in unicellular prokaryotes like *E. coli* and unicellular eukaryotes like yeast and in mammalian cells grown in tissue culture. Cloning can be done in vitro by polymerase chain reaction (PCR). In every case, the recombinant DNA must be taken up by the cell in a form in which it can be replicated and expressed. This is achieved by incorporating the DNA in a vector.

There are also various processes by which gene (DNA) transferred by bacteria in their host. These processes are described below:

1. Conjugation: Bacterial conjugation is the transfer of genetic information form one cell to another by physical contact. The ability to transfer DNA by conjugation is conferred by a conjugative plasmid, which is a self-transmissible element which encodes all the functions required to transfer a copy of itself to another cell by conjugation. The fertility (f) factor or

transfer factor, is an extra-chromosomal molecule that encodes the information necessary for conjugation. Conjugation involves two cell types: (a) Donors, which posses the F-factor and reterrred to as F+, and (b) Recipients, which lacks the F-factor and are referred to as F-. The R-factor contains the genes for the specialized pilus, called sex pilus, used in conjugation for other surface structures involved interactions with F- cells. The F- factor is self-transmissible once it is passed to an f=cell, the recipient cell becomes F+ and is able to pass the fertility factor to another F- cell. This is the means by which bacteria acquire multiple resistance to antibacterial agents. F- Cells receive chromosomal fragments, the size which depends on the time conjugation allowed to persist. The limiting factor for gene transfer is the stability of the bond between the sex pilus and the pilus receptor.

Once contact has been established, the DNA of conjugative plasmid is mobilized. Only one strand of DNA is transferred. In the recipient cell, the single- strand DNA is used as a template to generate double stranded molecule. In the donor cell, the remaining single strand also used as a template to replace the transferred strand. Thus, conjugation is thus semi-conservative process. One strand of F factor DNA moves in to recipient cell. An F' factor can conjugate. The recipient becomes F', and information passed across is part chromosome and part plasmid.

2. Transformation: It involves the uptake of naked DNA from the surrounding medium by a recipient cell and the recombination of genetic elements which change the genotype of the recipient cell.

Example: The transformation process was first demonstrated in 1928 by **Frederick Griffith**. Griffith experimented on ***Streptococcus pneumoniae***, a bacterium that causes pneumonia. When he examined colonies of the bacteria on petri plates, he could tell that there were two different strains. The colonies of one strain appeared smooth. Later analysis revealed that this strain has a polysaccharide capsule and is virulent, that it, it causes pneumonia. The colonies of the other strain appeared rough. This strain has no capsules and is a virulent. When Griffith injected living encapsulated cells into a mouse, the mouse died of pneumonia and the colonies of encapsulated cells were isolated from the blood of the mouse. When living non-encapsulated cells were injected into a mouse, the mouse remained healthy and the colonies of non-encapsulated cells were isolated from the blood of the mouse. Griffith

then heat killed the encapsulated cells and injected them into a mouse. The mouse remained healthy and no colonies were isolated. The encapsulated cells lost the ability to cause the disease. However, a combination of heat-killed encapsulated cells and living non-encapsulated cells affected the mouse pneumonia and colonies of living encapsulated cells were isolated from the mouse. How can a combination of these two strains cause pneumonia when either strand alone does not cause the disease? The answer is process of transformation.

The living non-encapsulated cells came into contact with DNA fragments of the dead capsulated cells. The genes that code for thr capsule entered some of the living cells and a crossing over event occurred. The recombinant cell now has the ability to form a capsule and cause pneumonia.

Process of transfer

1. Reversible association of DNA to the cell wall is mediated by an ionic interaction between DNA and the cell wall of competent organism. This type of association occurs in bacteria all the time, but if the cell is not competent the association is tenuous and the DNA is released and adsorbed elsewhere. Artificial competence can be induced by treating bacteria with calcium chloride. Calcium chloride alters cell membrane permeability, enabling the uptake of DNA by cells that are normally incapable of DNA adsorption.

2. Reversible association of the DNA and the inner cell membrane is established following transport of the DNA through the cell wall.

- Resistance to entracellular DNA are occurs as a consequence of conformational changes that take place after the DNA binds irreversibly to the cell membrane.
- Entry of DNA into cytoplasm- DNA enters the cytoplasm as a single strand.
- Integration in chromosomal DNA requires homology regions and involves displacement of one chromosomal strand, recombination of the invading strand, elimination of the remaining chromosomal segment, and duplication of the invading strand.

3. Transduction: In transduction, DNA is transferred from one cell to another by means of bacterial viruses, also known as bacteriophages. Bacteriophage can interact with bacteria intwo ways:

i) Virulent (lytic) infection eventually destroys the host bacterium.

ii) Template (lysogenic) infection is characterized by the integration of viral DNA into bacterial chromosome. The bacteria acquires a new set of genes: those of integrated phages (prophages)

Transduction can occur in two ways:

A. Generalized transduction: In generalized transduction, chromosomal or plasmid DNA accidentally become packaged into phage heads instead of the phage genome. GT can be used for mapping the bacterial chromosome, following the same principles involved in mapping by transformation. Properties of generalized transducing particle. They carry all host DNA or plasmid DNA, but no phage DNA. They can't replicate where these viruses infect another host cell, they inject purely chromosomal DNA from their former hosts. They are no functional viruses, just vessel carry in a piece of bacterial DNA. The generalized transducing phage can carry any part of the host chromosome

B. Specialized transduction: It takes place when a prophage contained in lysogenized bacterium replicates. Just as F1 plasmid are generated, a specialized transducing virus is generated when the cutting enzyme make a mistake. Properties of specialized transducing particle. They can't replicate

They carry hybrid DNA i.e. part phage DNA and part bacterial chromosome DNA. Unlike generalized transduction, specialized transduction is not a good mapping tool.

9.5.2 Enzymes Involved in Gene Transfer

Various enzymes involved in gene transfer for several functions. These have been isolated from prokaryotes which can be used to modify DNA and/or RNA in vitro reactions. Some of the important enzymes are described below:

Nulease: There are two types of nucleases viz. Exonuclease and Endonuclease. Exonucleases cleaves nucleotide one at a time from the end of a nucleic acid. Endonucleases cleaves bonds within a contiguous molecule of nucleic acid. Restriction

enzymes- Endonucleases:- cleave dsDNA,- do not cleave ssDNA or RNA - Each recognizes a specific nucleotide sequence, often palindromic. Palindromic is a state where both strands have the same nucleotide sequence but in anti-parallel directions 1.

II. RNases: Endonucleases cleave at specific ribonucleotides. RNAse A cleaves ssRNA at pyrimidine nucleotides B. RNase T1 cleaves ssRNA at G nucleotides.

III. DNA polymerases: They synthesize DNA polymerizes requirement for all DNA polymerases -Template strand (DNA or RNA). Primer with a free 31-OH group.

1. DNA polymerase (Holoenzyme) - The common source of DNA Polymerases is *E.coli*.

2. Klenow: It is on *E.coli* DNA pol I with 51→ 31 exonuclease activity removed. Its activity include: 51 → 31 polymerase.

T4 DNA polymerase: It is obtained from T4 plasmid infected E.coli . It has similar activities as to that of klenow except that 31→51= exonuclease activity is 200 α. It is used for end labeling of recessed

T7 DNA polymerase: It is obtained from T 7 –infected *E.coli* - It is highly possessive 51 →31 polymerase activity. It has no 31→51 exonuclease activity, its activity is 51→31 polymerase, it is used for DNA sequencing using the dideoxy system

Tag DNA polymerase: It is obtained from a thermophilic bacterium found in hot springs called thermos aquatic. It is heat stable polymerases that will synthesis DNA at elevated temperatures. It is used for: DNA synthesis in PCR reactions, Dideoxy DNA sequencing through regions of high 20 structures (i.e, regions destabilized by high temperature. Cyclic DNA sequencing of tow abundance DNA

Reverse transcriptase: It is obtained from either a. Arian myeloblastosiv virus or b. Moloney murine levkemia virus - it is an RNA dependent DNA polymerase - its activity include: 51 → 31 DNA polymerase and exoribonvclease specifically degrades RNA in a DNA: RNA hybrid. It is used to transcribe first cDNA in cDNA cloning.

IV.RNA polymerases: They are isolated from various bacteriophages including Phage SP6, Phage T7, Phage T3. Its activity is to synthesize ssRNA from a DNA template. Its uses include to synthesize sequence specific RNA probes which is used for labeling 5^1 ends of

synthetic oligonuceotides and Phosphorylating synthetic linkers and other synthetic DNA fragments lacking a 31- prior to ligation

V. Alkaline phosphatase: It is produced from either Bacteria (BAP) or calf intestine (CIAP), Its activity is removing the 5^1- phosphate from DNA or RNA. Its uses include remove 5^1 – phosphate from DNA prior to labeling, removing 5^1- phosphate from vector, DNA to prevent self-ligation during and cloning.

9.6 Usefulness of Transfer of Genes for Humankind

Transfer of genes deals with industrial scale production of biopharmaceuticals and biological using genetically modified microbes, fungi, plants and animals. Usefulness of transfer of genes include **therapeutics, diagnostics, and genetically modified crops for agriculture, processed food, bioremediation, waste treatment**, and **energy production**. The important applications of transfer of genes have been discussed below:

1) In Agriculture: Gene transfer is very useful in the field of agriculture. The gene transfer provides different plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). Genetic Modified plants have been useful in many ways.

- Gene transfer made plants more tolerant to environmental stresses such as cold, drought, salt, temperature. Therefore, plants can survive on different environmental conditions.
- Gene transfer provides such variety of plants which are not depend on chemical pesticides. These plants are pest-resistant crops.
- Gene transfer helped to reduce post harvest losses.
- Increased efficiency of mineral usage by plants.
- Genetic modified plants enhanced nutritional value of food. For example Vitamin 'A' enriched rice i.e. Golden Rice.

Bt Cotton: Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as lepidopterans, coleopterans (beetles) and dipterans (flies, mosquitoes). *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein. The Bt toxin protein exist as inactive *protoxins* but once

an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut. The activated toxin binds to the surface of midgut epithelial cells and creates pores that cause cell swelling and lysis and eventually cause death of the insect.

Specific Bt toxin genes isolated from *Bacillus thuringiensis* and incorporated into the several crop plants such as cotton. The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group specific. The toxin is coded by a gene named cry. There are a number of them, for example, the proteins encoded by the genes *cryIAc* and *cryIIAb* control the cotton bollworms, that of *cryIAb* controls corn borer.

2) In Medical Science: The gene transfer processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs. Further, the gene transfer do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources. At present, about 30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

3) Genetically Engineered Insulin: Management of adult-onset diabetes is possible by taking insulin at regular time intervals. Now, imagine if bacterium were available that could make human insulin. Suddenly the whole process becomes so simple. You can easily grow a large quantity of the bacteria and make as much insulin as you need.

4) Gene Therapy: If a person is born with a hereditary disease, gene therapy is an attempt to cure such types of disease. Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease.

5) Transgenic Animals: Animals that have had their DNA manipulated to possess and express foreign genes are known as transgenic animals. Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95% of all existing transgenic animals are mice.

(i) Normal physiology and development: Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as insulin-like growth factor. By introducing genes from other species that alter the

formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

(ii) Study of disease: Many transgenic animals are designed to increase our understanding of how genes are responsible for development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models have been developed for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis etc.

(iii) Biological products: Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA which codes for a particular product such as human protein used to treat emphysema (disease of lung). Similar attempts are being made for treatment of phenylketonuria and cystic fibrosis. In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk. The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.

(iv) Vaccine safety: Transgenic mice are being developed for use in testing the safety of vaccines before they are used on man. Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

(v) Chemical safety testing: The procedure is the same as that used for testing toxicity of drugs. Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

Summary

- Chromosomes are present in the nucleus of the cell. These are thread like structures. The numbers of chromosomes are fixed in a species for example human being has 23 pairs chromosomes, rat has 21 pairs chromosomes, cat has 19 pairs chromosomes.

- A chromosome is an organized structure of DNA and protein that is found in cells. A chromosome is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. The word chromosome derived from the Greek chroma which means color and soma which means body.
- On the basis of Position of Centromere chromosomes may be Metacentric , Sub-Metacentic, Acrocentric Chromosome, Holokinetic Chromosome.
- On the basis of number of Centromere these may be Acentric Chromosome (A chromosome without centromere. Such chromosome remains as laggard during cell division and is eventually lost), Monocentric Chromosome (A chromosome with one centromere. It represents normal type of chromosomes), Dicentric Chromosome.
- A gene is basic physical and functional unit of heredity. Genes are responsible for various characters in organisms. For example eye's colour, hair type, sex determination and some other characters depend on genes. Genes are typically 103 - 104 base pairs in size although they can be much larger. For example, the human dystrophin gene is 2 x 106 base pairs.
- Benzer (1955) divided the gene into recon, muton and cistron which are the units of recombination, mutation and function within a gene. Several units of this type exist in a gene. In other words, each gene consists of several units of function, mutation and recombination. The fine structure of gene deals with mapping of individual gene locus.
- There are various properties of genes which are forms, location, status, number, sequence, expression, change in Form, exchange of Genes, duplication, function, segregation, interaction, linkage.
- On the basis of different aspects genes may be dominant genes, recessive genes, epistatic, hypostatic, major, minor lethal genes, semilethal genes, sub-vital genes, vital genes, nuclear and plasma genes, normal genes and Jumping genes, split genes and Pseudo genes, sex linked genes, Sex limited genes and Sex influenced

genes, regulator genes, operator genes, promotor genes, Structural genes, mutable genes, Mutator genes and Antimutator genes.

- Plasmid is an extra chromosomal, self replicating and double stranded closed and circular DNA molecules present in the bacteria. It is small, circular pieces of DNA.
- There are various vectors such as Cosmid Vectors, phagemid Cloning Vectors, shuttle Vector, F-plasmid, Col plasmid, Suicide plasmids and virulence plasmids.
- Gene transfer describes the introduction of genetic information into a cell from another cell. This process occurs naturally in both bacteria and eukaryotes. Bacteria reproduce by the process of binary fission. In this process, the chromosome in the mother cell is replicated and a copy is allocated to each of the daughter cells.
- The important steps of gene transfer are DNA manipulation outside of cells, DNA transfer to recipient cell (vector), DNA manipulation within the recipient cell, Making a Recombinant DNA.
- Technique of Transfer of gene is very useful to humankind it is used in agriculture, in medicine and in production of transgenic plants and animals.

Terminal Questions

1 (a) Fill in the blank spaces with appropriate words.

A chromosome is an organized structure of ……..and …..that is found in cells. A chromosome is a single piece of coiled …….containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. The word ……..derived from the Greek …….which means ……and …….which means…….. This name is due to their property of being very strongly stained by particular dyes. Chromosomes vary widely between different organisms. The DNA molecule may be circular or linear, and can be composed of 10,000 to 1,000,000,000 nucleotides. Each chromosome has a constriction point called the……, which divides the chromosome into two sections, or ……..The short arm of the chromosome is labeled the …….. The long arm of the chromosome is labeled the …….. The location of the ……..on each chromosome gives the chromosome its characteristic shape, and can be used to help describe the location of specific genes.

2 (a) Define chromosomes.

2 (b) Write about the structure of chromosomes.

3 (a) Describe the properties and functions of genes.

3 (b) Explain different types of plasmids/vectors.

3 (c) Describe the classification of genes.

4 (a) Write about properties of plasmids

5 (a) Discuss the transfer of genes.

5 (b) Write about steps involved in transfer of genes.

5 (c) Which enzymes are involved in gene transfer? Explain

6 (a) Fill the blank spaces with appropriate words.

…….is an extra……., self replicating and double stranded closed and circular ……..molecules present in the…….. It is small, circular pieces of DNA. It …….independently of the …….cell. Plasmids have revolutionized molecular biology by allowing investigators to obtain many copies of custom DNA molecules. In addition to bacterial chromosome, bacterial cells normally contain genetic elements in their cytoplasm. These genetic elements exist and replicate separately from the chromosome and are called plasmids. The very existence of plasmids in bacterial cytoplasm was revealed by …….in the year 1952 while working on ……..process in bacteria. ……coined the term …….to refer to the transmissible genetic elements that were transferred from one bacterial cell to another and determined the maleness in bacteria.

6 (b) who discovered chromosomes (Nageli and Benden, Mendel and Morgan, Clarke and Lee)

6 (c) The term "Plasmid" was given by (Joshua Lederberg, Arne Nees, Morgan, Nageli)

6 (d) which is property of plasmid (Replicate independently/Species Specific/Act as episome/All of the above)

6 (e) Describe the usefulness of gene transfer to humankind?

7 (a) Discuss about plasmids in ecosystems.

7 (b) Give the similarities and dissimilarities between Chromosomes and genes.

7 (c) Discuss the types of chromosomes.

Answers

1 (a) DNA, protein, DNA, *chromosome,* chroma, color, soma, body, centromere, arms, p arm, q arm, centromere

2 (a) See section 9.2.; 2 (b) See section 9.2.1.

3 (a) See section 9.3.2; 3 (b) See section 9.4.2; 3 (c) See the section 9.3.3; 4 (a) See section 9.4.3; 5 (a) See the section 9.5; 5(b) See the section 9.5.1; 5(c) See section 9.5.2.

6 (a) Plasmid, chromosomal, DNA, bacteria, replicates, host, Lederberg, conjugation, Lederberg, plasmid; 6 (b) Nageli and Benden; 6 (c) Joshua Lederberg; 6 (d) All of the above; 6 (e) See the section 9.6.

7 (a) See the section 9.4.4; 7(b) See the section 9.3.2 (Table-1); 7(c) See the section 9.2.2

Unit 10 Xenobiotic and recalcitrant compounds

Unit Structure

10.0 Learning Objectives

After studying this unit you will be able to understand:

- Meaning, sources, transfer and interaction of xenobiotics
- About the classification of xenobiotics
- Effects of Xenobiotics
- How xenobiotics stabilized in disturbed ecosystems
- What are recalcitrant compounds?
- About sources, transfer, interaction of recalcitrant compounds
- How xenobiotics stabilize in ecosystems?

10.1 Introduction

As you know that our environment is made up of air, water, soil, plants, microbes and animals. You must be aware about the environmental pollution. A huge number of pollutants (agents which cause pollution) are disposed into the environment every day due to anthropogenic activities. The number of these pollutants is still increasing rapidly. Industrial and agricultural activities can require the intensive use of organic, inorganic and radioactive compounds. A combination of extensive use and indiscriminate release of xenobiotics of

different chemical structure and complexity into the environment cause substantial ecosystem stress. The presences of the compounds that are resistance to degradation are of concern because of their toxicity and biomagnification. Biomagnification is the process in which toxic chemicals increased in each trophic level. Xenobiotics are chemicals which may be accidently ingested or taken as drugs. They are found in organisms but not expected to be produced or present in them, or they are chemicals found in much higher concentration than usual. Drugs such as antibodies are xenobiotics in humans because neither human body produced them nor its a part of the normal diet. Natural products cannot become xenobiotics if they are taken up by another organism such as uptake of natural human hormones, by fish found downstream or sewage treatment plant out falls or the chemical defenses produced by some organisms as protection against predators. Xenobiotics are often used in the contest of pollutants such as dioxans and poly chlorinated biphenyls and their effect on biota because they are understood as substance foreign to an entire biological system. In this unit you will learn about various aspects of xenobiotics and recalcitrant compounds.

10.2 Xenobiotics

The term xenobiotic is derived from the Greek words **xenos** which means foreigner, stranger and **Bios** means **life**, **tikos** means **on**). A **xenobiotic** is a chemical substance found within plant or animal that is not naturally produced. It can also cover substances that are present in much higher concentration than are usual.

The term **xenobiotics** used in the context of pollutants such as dioxins and polychlorinated biphenyl. Xenobiotics are understood as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans. Xenobiotics may be grouped as hydrocarbons, carcinogens (cancer causing agents), drugs, environmental pollutants, food preservatives and food additives and pesticides.

Some xenobiotics substances are resistant to degradation. Xenobiotics such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and trichloroethylene (TCE) accumulate in the environment. These is because of their recalcitrant properties and xenobiotics have become an environmental issue due to their toxicity. This occurs particularly in the subsurface environment and water sources, as well

as in biological systems, having the potential to impact human health. Xenobiotics may be synthetic organochlorides such as plastics and pesticides, or naturally occurring organic chemicals such as polyaromatic hydrocarbons (PAHs) and some fractions of crude oil and coal.

Microorganisms are able to adapt to xenobiotics introduced into the environment through horizontal gene transfer, in order to make use of such compounds as energy sources. This process can be further altered to manipulate the metabolic pathways of microorganisms in order to degrade harmful xenobiotics under specific environmental conditions. Mechanisms of bioremediation include both genetically engineering microorganisms and isolating the naturally occurring xenobiotic degrading microbes.

Xenobiotics may be limited in the environment and difficult to access in areas such as the subsurface environment. Many xenobiotics produce a variety of biological effects, which is used when they are characterized using bioassay. Before they can be registered for sale in most countries, xenobiotic pesticides must undergo extensive evaluation for risk factors, such as toxicity to humans, ecotoxicity, or persistence in the environment. For example, during the registration process, the herbicide, cloransulam-methyl was found to degrade relatively quickly in soil.

10.2.1 Sources And Classification Of Xenobiotics

Sources: The major sources of xenobiotics are food additives such as colourings, flavours, preservatives, etc. The other sources are **fungicides, pesticides, herbicides, antibiotic** or **hormone residues, various other drugs and environmental pollutants** such as smoke produced by **automobiles, brick-kilns, industries, crackers** and fireworks used on festive occasions. Xenobiotic compounds are man-made chemicals that are present in the environment at unnaturally high concentrations. The xenobiotic compounds are either not produced naturally, or are produced at much lower concentrations than man.

There are various sources of xenobiotics which are given below:

1. Fungicides: Fungicides are the chemicals which are used to kill or destroy the harmful fungi. Fungicides have various negative impacts and may contain xenobiotics. There are various fungicides such as **azithrim, aureofungin, benomyl, chlorfenzole, dichlone,**

etridiazole etc. When these fungicides applied in agriculture field they may reach up to human body or other non-target species and act as xenobiotics.

2. Pesticides: Pesticides are also important source of xenobiotics. As you know pest is any biological component which destroy or damage the crop. Green revolution emphasized on excessive use of fertilizers and pesticides in agricultural land. When pesticides reach in body of human body they act as xenobiotics. In fact, they can also destroy other useful insects and other species. When these pesticides transfer into human body they can cause serious illness in human. Some important pesticides are **Isoproturon, benimyl, bifenthrin, cypermethrin, dichlorvos** etc.

3. Environmental pollutants: Various environmental pollutants also acts as xenobiotics. These are released by the industries. Environmental pollutants which act as xenobiotics are arsenic, cadmium, chromium etc.

4. Pharmaceutical drugs: Pharmaceutical industries are also important sources of the xenobiotics. These are chemicals used for the alteration, diagnosis, prevention and treatment of disease, health conditions or structure/function of the human body. Some pharmaceutically active compounds can enter the environment by one route or another as the parent compound or as pharmacologically active metabolites. Drugs are developed with the intention of having a beneficial biological effect on the organism to which they are administered, but many such compounds all too often pass into the environment. Nearly all categories of pharmaceuticals including pain killers, antibiotics, anticonvulsant drugs, Beta-blockers, blood lipid regulators, X-ray contrast media, cytostatic drugs, oral contraceptives, and veterinary pharmaceuticals among many others have been found in the environment. Pharmaceutically active compounds can be entered into the environment in two main ways; direct and indirect. Indirect sources are pharmaceutically active compounds that have performed their biologically intended effect and are passed onto the environment in either their complete or a modified state. These compounds can be discharged directly by manufacturers of the pharmaceuticals or effluent from hospitals. However with increasing regulation by local, state and federal regulating agencies, direct discharge is becoming much less of an issue.

There are also several indirect sources of pharmaceutically active compounds into the environment. One common indirect source of pharmaceutically active compounds into the environment is the passing of antibiotics, anesthetics and growth promoting hormones by domesticated animals in feacal matter. This is often stored in large pits before being pumped and applied to fields as fertilizers where many of the pharmaceutically active compounds can be washed away by rainfall to aquatic environments. Family pets can also be an indirect source of pharmaceutically active compounds into the environment. Most of the pharmaceutically active compounds in the environment however come from human sources. A direct human source is leachate from a landfill. Often the pharmaceuticals that are located in landfills are found in their original, most chemically active state.

Most pharmaceuticals are administered and passed through the human body in one of three ways:

1) Metabolized partially or completely within the body and made inactive.

2) Partially metabolized and passed through the system.

3) Passed through the body unmodified. In any manner pharmaceutically active compounds are then passed to sewage treatment plants, where facilities are designed to break down natural human waste by microbial degradation. However, many pharmaceutically active compounds are of very complex structure and are incompletely broken down in sewage treatment plants before they are passed into the environment. Common pharmaceutically active compounds found in the environment are analgesics (Acetaminophen, Acetylsalicylic Acid, Diclofenac, Codeine, Ibuprofen), Antibiotics (Macrolide Antibiotics, Sulfonamides, Fluoroquinolones, Chloramphenicol, Tylosin, Trimethoprim, Erythromycin, Lincomycin, Sulfamethoxazole, Trimethoprim), Anticonvulsant (Carbamazepine and Primidone), Beta-blockers (Metoprolol, Propanolol, Betaxolol, Bisoprolol, Nadolol) X-ray media (Iopromide, Iopamidol, Iohexol, Diatrizoate), Cytostatics (Cyclophosphamide, Mycophenolic acid, Ifosfamide, Bicalutamide, Epirubicin), Steroids and hormones (17α-ethinylestradiol, Mestranol, 19-norethisterone).

Classification Of Xenobiotics: The broad categories of Xenobiotic pollutants are summarized in Fig-1 also discussed below:

i) Recalcitrant halocarbons: The carbon–halogen bond in them is very stable. Cleavage of this bond is not exothermic and requires a substantial energy input as it is an endothermic reaction. There seems to be little prospect for microorganisms ever to evolve a capability for utilization of extensively halogenated carbon compounds as grow material none the less some cometabolism and photodegradation may occur in them.

ii) Haloalkyl propellants and solvents: Chloro benzenes comprise major industrial solvents. Their aerobic bidegrability declines with the number of halo-substituents but the same halogenated aromatics are dechlorinated anaerobically. On the other hand Pseudomonas and Alcaligenes use the dioxygenase to produce chlorocatechols from aerobic biodegradation of trichloro and tetrachlorobenzenes. The highly chlorinated chlorophenols as pentachlorophenol (PCP) are used as preservatives for wood and canvas. Some chlorophenols are used to make pesticides.

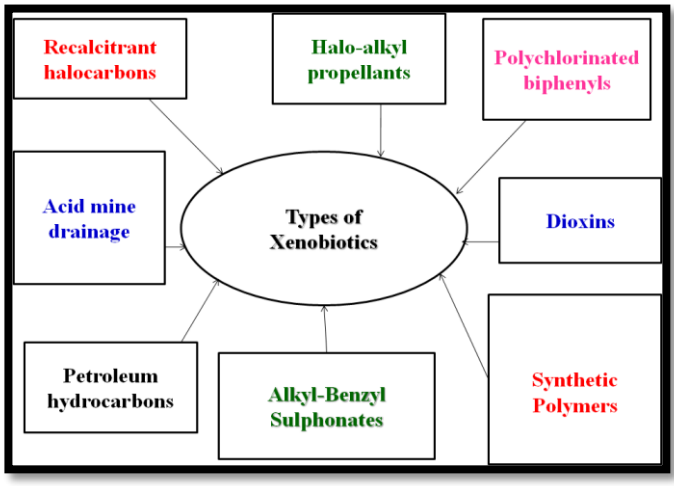


Fig-1: Showing different types of Xenobiotics

iii) Polychlorinated biphenyls (PCBs) and dioxins: The PCBs are mixtures of biphenyls with 1 to 10 chlorine atoms per molecule. Their structures are almost like DDT and are also known to accumulate in higher trophic levels. Although PCBs are relatively resistant to biodegradation yet some microorganisms have be isolated which can transform them. PCBs can be biodegraded aerobically using white rot fungus Phanerochaete, by Acinetobacter and by Alkaligenes and anaerobically by reductive dehalogenation. The degradation of PCBs is by cometabolism and can be increased by adding less chlorinated analogs like dichlorophenyl. Dioxins are highly toxic compounds and produced as a by-product in some manufacturing processes.

iv) Alkyl benzyl sulphonates: They are main components of anionic detergents. They are surface active with polar sulphate and nonpolar alkyl end in their molecule. Due to their characteristic of emulsification of fatty substances and thereby cleaning occurs while these molecules make a monolayer around lipophilic droplets or particles. Their molecules orient with their nonpolar end towards the lipophilic substance and the sulphonate end towards water. Nonlinear alkylbenzyl sulphonates are recalcitrants and resistant to biodegradation and cause foaming in the rivers in plenty.

v) Petroleum hydrocarbons: Most xenobiotic pollutants are substituted or modified hydrocarbons. Their short chains are toxic to organisms but they evaporate quickly from the oil slicks. However, very long chain alkanes are resistant to biodegradation. Increasing chain length makes alkanes to exceed molecular weight of 500 and consequently alkanes stop to serve as carbon source. Moreover, branching often reduces the rate of biodegradation because tertiary and quaternary carbon atoms interfere with degradation or at all do not degrade. Aromatic compounds particularly of condensed polymer type are degraded at a much slow rate in comparison to alkanes. The alicyclic compound frequently do not serve as the sole carbon source for microbial growth until and unless they are provided with long aliphatic side chain but they cannot be degraded through cometabolism using 2 or more strains that exhibit cooperative mechanisms because of their complementary metabolic ability.

Xenobiotics further may be two types viz. **Exogenous and endogenous.** Exogenous**-** The foreign molecules which are not normally ingested or utilized by organism but they gain entry through dietary food stuffs, or in the form of certain medicines/drugs used for a therapeutic cause or are inhaled through environment. Example: Drugs, food additives, pollutants, insecticides, chemical carcinogens etc. Endogenous**-** Though they are not foreign substances but have effects similar to exogenous xenobiotics. These are synthesized in the body or are produced as metabolites of various processes in the body. Example: Bilirubin, Bile acids, steroids, Eicosanoids and certain fatty acids.

10.2.2 Transfer of Xenobiotics in Human Body

As you know xenobiotics are chemicals found in organisms, but not expected to be produced or present in them; or they are chemicals found in higher concentrations than normal. Xenobiotics transfer in human body by the skin absorption, inhalation and ingestion.

Inhalation: For most xenobiotics specially which are present in polluted, inhalation is the major route of entry. Once inhaled, xenobiotics are either exhaled or deposited in the respiratory tract. If deposited, damage can occur through direct contact with tissue or the chemical may diffuse into the blood through the lung-blood interface. Upon contact with tissue in the upper respiratory tract or lungs, xenobiotic may cause health effects ranging from simple irritation to severe tissue destruction. Substances absorbed into the blood are circulated and distributed to organs that have an affinity for that particular chemical. Health effects can then occur in the organs, which are sensitive to the toxicant.

Skin (or eye) absorption: Skin (dermal) contact can cause effects that are relatively innocuous such as redness or mild dermatitis; more severe effects include destruction of skin tissue or other debilitating conditions. Many xenobiotics can also cross the skin barrier and be absorbed into the blood system. Once absorbed, they may produce systemic damage to internal organs. The eyes are particularly sensitive to chemicals. Even a short exposure can cause severe effects to the eyes or the substance can be absorbed through the eyes and be transported to other parts of the body causing harmful effects.

Ingestion: Xenobiotics that accidentally get in to the mouth and are swallowed do not generally harm the gastrointestinal tract itself unless they are irritating or corrosive. Xenobiotics that are insoluble in the fluids of the gastrointestinal tract (stomach, small, and large intestines) are generally excreted. Others that are soluble are absorbed through the lining of the gastrointestinal tract. They are then transported by the blood to internal organs where they can cause damage.

After absorption or inhalation or ingestion they come in contact of plasma membrane. There are five possible transfer processes of intestinal absorption of Xenobiotics; they are active transport, passive diffusions, Pinocytosis, filtration through “pores” and lymphatic absorption. There are number of factors which alter the rate of Xenobiotics absorption which include diet, motility of intestine, interference with gastro intestinal flora, changes in the rate of gastric emptying, age, and the dissolution rate. Xenobiotics are metabolised by biotransformation

or detoxification reactions and they are classified into phase one and phase two reactions. Phase one reactions include oxidations reaction, reduction, hydrolysis and phase two reactions include sulfation, acetylation, methylation and conjugation with glucronic acid, glutathione and glycine.

Xenobiotics must cross the intestinal epithelium, basement membrane and capillary endothelium before they reach the blood stream. Mammals do not absorb the Xenobiotics through any special transport processes but share the same transport processes which are used absorption of nutrients. There are five possible processes of Xenobiotics transport across the intestine. They are:

a. Active transport

b. Pinocytosis

c. Filtration through “pores”

d. Lymphatic absorption

e. Passive diffusion

a) Active transport: Active processes require cellular energy, food transfer of substrate across the intestine against higher concentration. The system exists mainly for transport of natural substances (aminoacids, sugar, or bile acids).

b) Pinocytosis: In this cell membrane forms invagination which finally close to form vesicles which contain fluid from outside the cell. Inside the cell the contents of vesicles are delivered to cytoplasm. In suckling animals this process of transport is used for macromolecules (antigenic peptide, an immune-active protein).

c) Filtration through pores: Both lipophilic and hydrophilic compounds may pass through holes in the cell membrane. Xenobiotics with molecular weight 100 may be absorbed through this process.

d) Lymphatic absorption: It is well known that dietary short chained fatty acids predominantly absorbed via the lymphatic system in minute droplets known as chylomicrons.These enter the thoracic duct and empty into the systemic venous blood;completly bypassing the liver. Eg: Para amino salicylic acid, tetracycline, DDT, benzpyrene and 3-methyl cholanthrene.

e) Passive diffusion: Passive diffusion is a major process for absorption of Xenobiotics. This process is not saturable and transfer is directly proportional to the concentration gradient and to the lipid –water partition coefficient of Xenobiotics. The higher the factors, faster the rate of diffusion, and when concentrations are the same on both sides of membrane, movement of Xenobiotics across the membrane stops. Absorption of structurally related chemicals occurs independently; co absorption does not alter absorption rate of either chemical. The extent of lipid solubility and the ionisation of Xenobiotics influence the rate of chemicals.

Factors affecting intestinal absorption of Xenobiotics: Factors such as diet, age, species, changes in the motility of the intestinal tract, interference with gastro intestinal content of microorganisms, changes in the rate of gastric emptying in either direction and dissolution rate of Xenobiotics can influence the intestinal absorption of xenobiotics.

Metabolism of Xenobiotics: Xenobiotics are metabolised by biotransformation or detoxification reaction. The compound that are detoxified include:

- Compounds accidently ingested like preservatives, food additives and adulterants
- Drugs taken for therapeutic purposes
- Compounds produced in the body and are to be eliminated. eg: Bilirubin and steroids
- Compounds produced by bacterial metabolism. eg: Amines produced by decarboxylation of aminoacid.Histidine to Histamine, Tyrosine to tyramine.

The transformation of specific xenobiotic can be either beneficial or harmful and perhaps both depending on the dose. A good example is biotransformation of acetaminophen, a commonly used drug to reduce pain and fever. It normally undergoes rapid biotransformation with the metabolite quickly eliminated in urine and faeces. Hence no toxicity is observed .The excess acetaminophen undergoes additional biosynthetic path way, which produces a metabolite that is toxic to the liver. Cytochrome P450 is the main enzyme involved in the biotransformation reaction pathway which produces a metabolite that is toxic to the liver.

Phases of detoxification reaction: Biotransformation reactions are usually classified as phase I and II reaction.

Phase I is an alteration of the foreign molecule, so as to add functional group which can be conjugated in phase II. Phase I reaction result in the formation of compounds with decreased toxicity (detoxification).Sometimes this may result in increased toxicity (entoxification) e.g.: methanol to formic acid. The phase I reactions include hydroxylation, oxidation, reduction, hydrolysis, dealkylation, epoxidation etc.

The products of metabolic transformation are either excreted directly or undergo further metabolism by phase II reaction. They involve conjugation with a conjugating agent, thus converting lipophilic drug to water soluble, easily excretable forms. Phase II reactions are sulfation, acetylation, methylation and conjugation with glucronic acid, glutathione and glycine[23]. In some instances products of phase II may further be metabolized by phase III reactions. Sometimes both phase I and II reactions are needed to detoxify a compound.

Phase III reactions are not very common .A typical example is further conjugation with glutathione. The Xenobiotics that enter the body are mostly drugs and they are detoxified by the enzymes concerned with drug metabolism. Induction of cytochrome P450 system may even produce unwanted effects in some persons. For example induction of ALA synthase by barbiturates will precipitate attacks in acute intermittent porphyria.

10.2.3 Interaction Of Xenobiotics With Community

Environmental contamination by xenobiotics is a global phenomenon as a result of human activities resulting from rise in urbanization and **industrialization**. There are numerous sources of xenobiotics ranging from pharmaceuticals to **agriculture**. In recent time, the demand for pharmaceuticals versus population growth has placed the public at risk. In addition, the making of unlawful drugs has led to the discharge of harmful carcinogens into the environment. The release of these harmful pollutants results in numerous short- and long-term effects to the environment. Originally, the term xenobiotic comes from the Greek word **xenos**, which means **foreign** or **strange**, and 'bios', which means life. Xenobiotics are chemical compounds exhibiting **abnormal** structural characteristics. The presence of **antibiotic** drugs in the human body which may not be produced by the body itself nor is a normal part of diet is example of **xenobiotic**. At times, a natural substance can be defined as a xenobiotic if it found its way into humans or other animals.

There are different synthesized chemicals present in the environment which may have different interactions with the exposure to humans and the ecosystem. However, the details of these impacts are not adequately studied or understood. Among the different pharmaceutical substances, pharmaceutical active compounds are xenobiotic-based elements that entered the environment as the parent compound or as pharmacologically active metabolites. Pharmaceutical active compounds are considered as potentially toxic compounds that are largely used in agriculture and industry.

Bonjoko explained the interaction of xenobiotics and household care products in the environment. It includes:

- Through patient excretion
- Direct release into the wastewater system from manufacturing, hospitals or disposed through toilets and sinks
- Terrestrial depositions, i.e. irrigation with treated and untreated wastewater, sludge application to land, leaching from solid waste landfills
- Non-pharmaceutical industrial sources, i.e. plastic products
- Agricultural wastes such as herbicides, pesticides and fertilizers
- Through ageing infrastructures, i.e. synthetic compounds such as analgesics and antihistamines which were exposed in streams and rivers
- Drugs associated with plant health
- Herbal preparations and their interaction with the environment

10.2.4 Effects of Xenobiotics

There are various effects of xenobiotics on human and environment, the important effects of xenobiotics summarized in Fig-1 and also described below:

Effects of xenobiotics on Human: Xenobiotics have various health effects on human. The possible exposure pathway of endocrine disruptors in humans includes direct exposure at the workplace and via consumer products such as food, certain plastic, paints, detergents and cosmetics. They have also indirect exposure via the environment, viz. air, water and soil. Lead is regarded as being hepatotoxic (toxic to liver), while cadmium is a well-known nephrotoxic (toxic to nervous system). Health effects of Xenobiotics are irritation to the eye, nose and throat, injury to the central nervous system and kidney and cancer. Symptoms of xenobiotics toxicity include nausea, muscular weakness, headache and dizziness, whereas chronic exposure to certain xenobiotics could result in liver, kidney, endocrine and nervous system damage. Exposure to elevated levels of cyclodiene xenobiotics associated with improper use caused various symptoms, including headaches, dizziness, muscle twitching, weakness, tingling sensation and nausea. It is assumed that cyclodienes might cause long-term damage to the liver and the central nervous system. It is also acts as carcinogen.

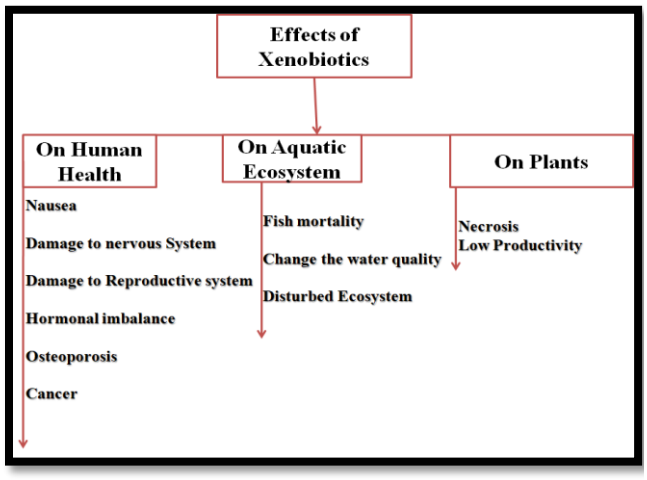


Fig-1: Showing various impacts of xenobiotics

Steroid receptors for oestrogens and androgen functions of the brain and the cardiovascular, the skeletal and the urogenital system are regulated by these hormones and can therefore be affected by xenobiotics. Xenobiotics have the potential to cause reduced quality of sperm, low ejaculate volume and high number of abnormal spermatozoa motility. Other effects may include testicular cancer and malformed reproductive tissue, viz. undescended testes, small penis size, prostate disease and other unrecognized abnormalities of male reproductive organ.

Bisphenol A, xenobiotic which is, binds to the local anaesthetic receptor site to block the human cardiac sodium channel. Xenobiotics can cause some female diseases including breast and reproductive organ tissue cancers, fibrocystic disease of the breasts, polycystic

ovarian syndrome, endometriosis, uterine fibroid and pelvic inflammatory diseases. Phthalates, a xenobiotic used in cosmetics like nail polish, are reported to affect the endocrine system and are being investigated for a link with infertility in women. Xenobiotics have been linked to impaired behaviour, mental, immune and thyroid functions in developing children. Others effects of xenobiotics may include osteoporosis, foetal growth and obesity. Children are most prone to environmental contaminants from foodstuff to drug and plastic toys.

Effects on Aquatic Ecosystem: Xenobiotics have various harmful impacts on aquatic ecosystem, significantly. Aquatic organisms are significant biological indicators of pollution. Pharmaceuticals are most often released back into the environment either in their original form or as metabolites. In humans, the main pathway is ingestion following excretion and disposal via wastewater. Municipal wastewater is the largest source of human pharmaceuticals. Hospital wastewater, wastewater from manufacturers and landfill leachates may contain significant concentrations of xenobiotics. Xenobiotics that are nondegradable in the sewage treatment plant (STP) are being released into treated effluents resulting in the contamination of rivers, lakes, estuaries and groundwater and eventually drinking water. There is also likelihood of contamination when sewage is applied in agriculture. In addition, drugs meant for animals enter the waterways during surface application for agriculture purposes and runoff and also via direct application in fish farming. Xenobiotics of environmental significance oftentimes have high production volume in addition to environmental persistence and biological activity, especially after long-term exposure. It has been observed that the increasing amounts of xenobiotics found in surface waters worldwide have raised concerns especially with respect to their effects on the aquatic flora and fauna. Among aquatic organisms, fish most often share drug targets with humans. Not much is known about the long-term effect of drugs in aquatic organisms. Diclofenac influences the expression of genes in fish and organ histology when exposed to a concentration of l µg/L of this drug. Streams and rivers have been identified to be exposed to combinations of different Xenobiotics. Antidiabetic and antihistamine diphenhydramines were observed to cause significant disruption to the biofilm community which is important to the ecosystem.

Biofilms are aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substances adhere to each other and or to a surface. Biofilms serve as the important food source for invertebrates that in turn feed larger animals like fish. The effects of diphenylamines on biofilm could therefore have repercussion for animals in stream food web such as insects and fish. The use of antidepressants disrupts the aquatic equilibrium by activating early spawning in some shellfish. Furthermore, propranolol and fluoxetine were observed to have deleterious effects on zooplankton and benthic organisms.

Dichlorodiphenyldichloroethylene induced eggshell thinning in birds is probably the best example of reproductive impairment causing several population declines in a number of raptor species in Europe and North America. Gradual exposure to the DDT complex as been linked to ovotestis in male western gulls. EDCs (endocrine disruptors) have negatively affected a variety of fish species exposed to effluents causing reproductive problems. Turtles have also been affected in a similar manner. Triclosan is a broad-spectrum antimicrobial compound that is contained in most of the cleaning products for the prevention of bacterial, fungal and mildew growth. Triclosan enters into water streams from domestic wastewater, leaking sewerage and sewage overflows. The continuous use of these antibiotics leads to the emergence of resistant bacteria that could diminish the usefulness of important antibiotics.

Effects on Animals: More commonly observed effects of xenobiotics are impaired reproduction and development in aquatic animals. A number of brain targets for xenobiotics present in environmentally relevant concentrations in surface waters had been identified from recent surveys. ***In mammals, field studies on Baltic grey and ringed seals and Wadden Sea harbour seals revealed that reproduction and immune function were impaired by PCBs (polychlorinated biphenyls).*** Mammals in the food chain that have the likelihood of being affected include the polar bear, rabbit and guinea pig. In Florida, due to a pesticide spill, alligators were found to have inhibited genital developments. Furthermore, the oestrogenic and androgenic effects observed have been linked to experimental studies with alligator eggs to the DDT complex.

Effects of Plants: Xenobiotics also affect the plants. The Xenobiotics cause necrosis, close the stomata of the plants. The low productivity of the crops also observed due to the xenobiotics. Xenobiotics also may reach the high trophic level through the plants by the process of biomagnification. The road side plants highly affected by the exposure of xenobiotics.

10.2.5 Stablization Of Xenobiotics In Disturbed Ecosystems

There are various bacteria which can stabilize and degrade the xenobiotics in different ecosystems. Species such as *Streptomyces, Pseudomonas, Achromobacter, Rhodococcus, Bacillus fusiformis* etc. can degrade naphthalene. *Pseudomonas* species, *Massillia* species, *Sphingobium*, *Mycobacterium*, *Brevibacillus, Sphingomonas* can degrade polycyclic aromatic hydrocarbon. Certain species of *Microbacterium, Martelella, Ochrobactrum, Rhodococcus opacus, Pseudomonas aeruginosa, Pseudomonas citronellolis* can degrade Anthracene. Petachlorophenol can be stabilized by the *Kocuria* species*, Comamonas testosteroni, Sphingobium* species, *Bacillus cereus* and *Sphingomonas chlorophenolica*. Chloroaniline can be degraded by *Acinetobacter baylyi, Delftia tsuruhatensis, Acinetobacter baumannii* and *Pseudomonas putida*. 1,2,4-trichlorobenzene can be stablized by *Pseudomonas utida* and *Bordetella* spceis. 2-chlorobenzoic acid can be degraded by *Pseudomonas, Enterobacter, Acinetobacter* and *Corynebacterium* spceis. Fluoranthene can be stablized by *Herbaspirillum chlorophenolicum*. Spcies of *Klebsiella, Bacillus, Diaphorobacter and Pseudoxanthomonas* degrade Pyrene. Many bacteria such as *Acgromobacter denitrificans, Arthrobacter, Agrobacterium, Ochrobatrum, Enterobacter, Rhodococcus* can stablize xenobiotics (Phthalate) in natural ecosystems.

Degradation of Xenobiotics: It is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, but it is not always possible. Bioremediation takes place by the following actions.

- **Detoxification:** Detoxification process includes the microbial conversion of toxic compound to a nontoxic one. Biodegradation involving detoxification is highly advantageous to the environment.

- **Activation:** Many xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous process and can lead to more pollution in nature.
- **Degradation:** In this process the complex compounds are degraded to simpler products which are generally harmless. This process takes place by the enzymatic action of microbes.
- **Conjugation:** The process of conjugation may include the conversion of xenobiotics to more complex compounds. This process is rarely observed in nature.

Types of Reactions in Bioremediation: Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

1) Aerobic bioremediation: Aerobic biodegradation involves the utilization of O_2 for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms. Two types of enzymes viz. **mono-oxygenases** and- **di-oxygenases** are involved in aerobic biodegradation. **Mono-oxygenases** can act on both aliphatic and aromatic compounds. Di-oxygenases oxidize aliphatic compounds.

2) Anaerobic bioremediation: Anaerobic biodegradation does not require oxygen. The growth of anaerobic microorganisms and consequently the degradation processes are slow. However, anaerobic biodegradation is cost- effective, since the need for continuous O_2 supply is not there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below.

Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dechlorination — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. Carboxylation and decarboxylation — toluene, cresol and benzoate.

Sequential Bioremediation: In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

Biodegradation of Hydrocarbons: Hydrocarbon are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of

microorganisms e.g. Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium and Nocardia.

Biodegradation of Aliphatic Hydrocarbons: The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are reclacitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride).

Biodegradation of Aromatic Hydrocarbons: Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas.

Biodegradation of Pesticides and Herbicides: Pesticides and herbicides are regularly used to contain various plant diseases and improve the crop yield. In fact, they are a part of the modern agriculture, and have significantly contributed to green revolution. The common herbicides and pesticides are propanil (anilide), propham (carbamate), atrazine (triazine), picloram (pyridine), dichlorodiphenyl trichloroethane (DDT) monochloroacetate (MCA), monochloropropionate (MCPA) and glyphosate (organophosphate). Most of the pesticides and herbicides are toxic and are recalcitrant (resistant to biodegradation). Some of them are surfactants (active on the surface) and retained on the surface of leaves.

Biodegradation of Halogenated Aromatic Compounds: Most commonly used herbicides and pesticides are aromatic halogenated (predominantly chlorinated) compounds. The bio-degradative pathways of halogenated compounds are comparable with that described for the degradation of non-halogenated aromatic compounds. The rate of degradation of halogenated compounds is inversely related to the number of halogen atoms that are originally present on the target molecule i.e. compounds with higher number of halogens are less readily degraded.

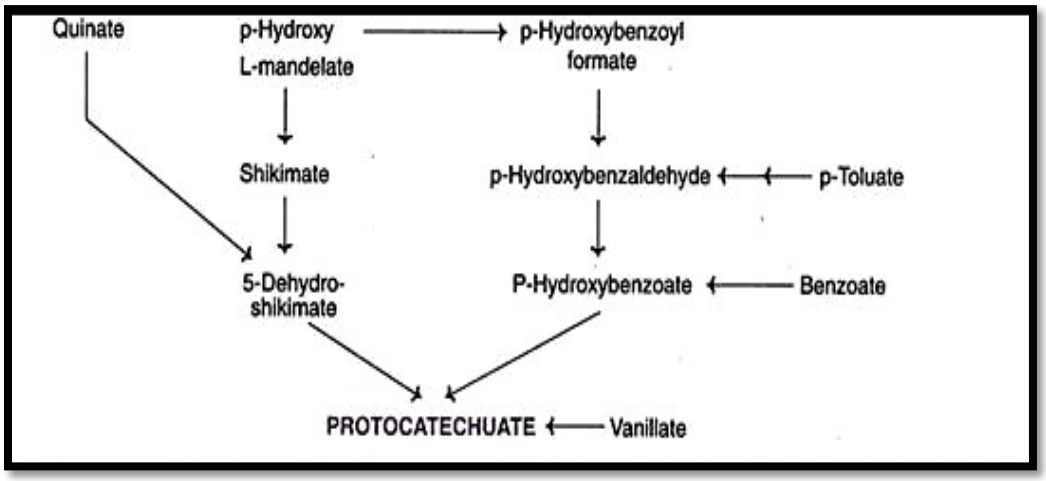


Fig-2: Bioremediation of xenobiotics to produce protocatechuate

Dehalogenation of halogenated compounds is an essential step for their detoxification. Dehalogenation is frequently catalysed by the enzyme di-oxygenase. In this reaction, there is a replacement of halogen on benzene with a hydroxyl group. Most of the halogenated compounds are also converted to catechol and protocatechuate which can be metabolised. Besides *Pseudomonas*, other microorganisms such as *Azotobacter, Bacillus* and *E. coli* are also involved in the microbial degradation of halogenated aromatic compounds.

Biodegradation of Polychlorinated Biphenyls (PCBs): The aromatic chlorinated compounds possessing biphenyl ring (substituted with chlorine) are the PCBs e.g. pentachlorobiphenyl. PCBs are commercially synthesized, as they are useful for various purposes as pesticides, in electrical conductivity (in transformers), in paints and adhesives. They are inert, very stable and resistant to corrosion. However, PCBs have been implicated in cancer, damage to various organs and impaired reproductive function. Their commercial use has been restricted in recent years, and are now used mostly in electrical transformers.

Genetic Engineering for More Efficient Bioremediation: Although several microorganisms that can degrade a large number of xenobiotics have been identified, there are many limitations in bioremediation:

1. Microbial degradation of organic compounds is a very slow process.
2. No single microorganism can degrade all the xenobiotics present in the environmental pollution.
3. The growth of the microorganisms may be inhibited by the xenobiotics.

4. Certain xenobiotics get adsorbed on to the particulate matter of soil and become unavailable for microbial degradation.

It is never possible to address all the above limitations and carry out an ideal process of bioremediation. Some attempts have been made in recent years to create genetically engineered microorganisms (CEMs) to enhance bioremediation, besides degrading xenobiotics which are highly resistant (recalcitrant) for breakdown. Some of these aspects are briefly described.

Genetic Manipulation by Transfer of Plasmids: The majority of the genes responsible for the synthesis of bio-degradative enzymes are located on the plasmids. It is therefore logical to think of genetic manipulations of plasmids. New strains of bacteria can be created by transfer of plasmids (by conjugation) carrying genes for different degradative pathways. If the two plasmids contain homologous regions of DNA, recombination occurs between them, resulting in the formation of a larger fused plasmid (with the combined functions of both plasmids). In case of plasmids which do not possess homologous regions of DNA, they can coexist in the bacterium (to which plasmid transfer was done). The first successful development of a new strain of bacterium (Pseudomonas) by manipulations of plasmid transfer was done by Chakrabarty and his co-workers in 1970s. They used different plasmids and constructed a new bacterium called as superbug that can degrade a number of hydrocarbons of petroleum simultaneously. United States granted patent to this superbug in 1981 (as per the directive of American Supreme Court). Thus, superbug became the first genetically engineered microorganism to be patented. Superbug has played a significant role in the development of biotechnology industry, although it has not been used for large scale degradation of oil spills.

10.3 Recalcitrant Compounds

The **recalcitrant** chemicals can resist biodegradation for various reasons and some are more **resistant** than others. The failure of a compound to be degraded or its slow biodegradation may be because of environmental and chemical factors. Many recalcitrant chemicals are toxic to some extent to some or most microbes. The examples include most **phenols** and fungicides. Concentration is a major factor in the fate of recalcitrant compounds in the environment. If the concentration is too high, problems of toxicity may **arise**. If a

chemical enters an **environmen**t with very few organisms in it, then the degradation will either be slow or will not occur. Pristine water in the upper reaches of a stream, ground water (in aquifers), and subsoil have very small microbial communities, thus the recalcitrant compounds entering these environments are degraded slowly. The presence of **acclimated organisms**, which are previously exposed to a target compound, can greatly accelerate biodegradation.

In environment any compound or molecule that persists in nature for long time and resist degradation called **recalcitrant compounds**. The persistence of man-made chemicals may be a necessary requirement for compounds designed specifically for technical use, or of compounds, like insecticides directed towards specific targets. In general, however, persistence of man-made chemicals in the natural environment is undesirable. Studies on degradation including both biotic and abiotic processes therefore occupy a central position in environmental hazard assessments.

The xenobiotic compounds may be recalcitrant due to one or more of the following reasons:

- They are not recognised as substrate by the existing degradative enzymes,
- They are highly stable, i.e., chemically and biologically inert due to the presence of substitution groups like halogens, nitro-, sulphonate, amino-, methoxy- and carbamyl groups,
- They are insoluble in water, or are adsorbed to external matrices like soil,
- They are highly foxic or give rise to toxic products due to microbial activity,
- Their large molecular size prevents entry into microbial cells,
- Inability of the compounds to induce the synthesis of degrading enzymes, and
- Jack of the perm-ease needed for their transport into the microbial cells.

Types of Recalcitrant compounds: The recalcitrant xenobiotic compounds can be grouped into the following 6 types:

(i) Halocarbons: These compounds contain different numbers of halogen (e.g., Cl, Br, F (fluorine), I) atoms in the place of H atoms. They are used as solvents (chloroform, $CHCl_3$), as propellants in spray cans of cosmetics, paints etc., in condenser units of cooling systems

(Freons, CCl_3F, CCl_2F_2, $CClF_3$, CF_4), and as insecticides (DDT, BHC, lindane etc.) and herbicides (dalapon, 2, 4-D, 2, 4, 5-T etc.). The C_1-C_2 haloalkanes like chloroform, freons etc. are volatile and escape into the atmosphere where they destroy the protective ozone (O_3) layer leading to increased UV radiation. Pesticides (herbicides, fungicides and insecticides) are applied to crops from where they leach into water bodies; many of them are subject to bio-magnification.

(ii) Polychlorinated biphenyls: These compounds have two covalently linked benzene rings having halogens substituting for H. PCB's are used as plasticisers, insulator coolants in transformers and as heat exchange fluids. They are both biologically and chemically inert to various degrees, which increases with the number of chlorine atoms present in the molecule. The recalcitrant nature of the above two groups of compounds is due to their halogenation and as well their cyclic structure (PCB's).

(iii) Synthetic Polymers: These compounds are produced as plastics, e.g., polyethylene, polystyrene, polyvinyl chloride etc., and nylons which are used as garments, wrapping materials etc. They are recalcitrant mainly due to their insolubility in water and molecular size.

(iv) Alkylbenzyl Sulfonates: These are surface-active detergents superior to soaps. The sulphonate (— SO_3^-) group present at one end resists microbial degradation, while the other end (non-polar alkyl end) becomes recalcitrant if its is branched, (resistance increases with the degree of branching). At present, alkylbenzyl sulphonates having non-branched alkyl ends are used; these are biodegraded by β-oxidation from their alkyl ends.

(v) Oil Mixtures: Oil is a natural product, has many components and is biodegradable, the different components being degraded at different rates. Biodegradation is able to handle small oil seepages. But when large spills occur the problem of pollution becomes acute. Oil is recalcitrant mainly because of its insolubility in water and due to the toxicity of some of its components.

(vi) Other Recalcitrant Compounds: A number of pesticides are based on aliphatic, cyclic ring structures containing substitution of nitro-, sulphonate, methoxy-, amino- and carbomyl groups; in addition, they also contain halogens. These substitutions make them recalcitrant.

Hazards from Xenobiotic Compounds: The xenobiotics present a number of potential hazards to man and the environment which are briefly listed below.

(i) Toxicity: Many xenobiotics like halogenated and aromatic hycrocarbons are toxic to bacteria, lower eukaryotes and even humans. At low concentrations they may cause various skin problems and reduce reproductive potential.

(ii) Carcinogenicity: Certain halogenated hydrocarbons have been shown to be carcinogenic.

(iii) Many xenobiotics are recalcitrant and persist in the environment so that there is a build up in their concentration with time.

(iv) Many xenobiotics including DDT and PCB's are recalcitrant and lipophilic; as a consequence they show bioaccumulation or bio-magnification often by a factor of $10^4 – 10^6$.

General Features of Biodegradation of Recalcitrant compounds: Since recalcitrant compounds consist of a wide variety of compounds, their degradation occurs via a large number of metabolic pathways.

Degradation of recalcitrant compounds generally occurs as follows:

(i) An oxygenase first introduces a hydroxyl group to make the compound reactive,

(ii) The hydroxyl group is then oxidised to a carboxyl group,

(iii) The ring structure is opened up (in case of cyclic compounds),

(iv) The linear molecule is degraded by β-oxidation to yield acetyl CoA which is metabolised in the usual manner. For example, an n-alkane is oxidised as follows.

Similarly, an alicyclic hydrocarbon, e.g., cyclohexane, is oxidised as follows:

(i) First an oxygenase adds an —OH group in the ring,

(ii) Then another oxygenase forms an ester in the form of a lactone,

(iii) Which is then hydrolysed to open the ring structure to yield a linear molecule.

In both these oxidations mono-oxygenases are involved which add oxygen to a single position in the molecule. In contrast, oxidation of benzene ring may involve a di-oxygenase which adds oxygen at two positions in the molecule in a single step.

Both mono- and di-oxygenases are of a variety of types: some react best with short chain alkanes, while others act on cyclic alkanes. But these enzymes are not very specific and each enzyme oxidise a limited range of compounds. Thus xenobiotics are degraded by a wide variety of microorganisms, each of which degrades a small range of compounds. Frequently, oxidation of xenobiotics involves cytochrome P_{450} or rebredoxin. In addition, the halogens and/or other substituent groups are either modified or removed usually as one of the initial reactions or sometimes it is achieved later in the process.

Summary

In this unit we have discussed various aspects of xenobiotics and recalcitrant compounds. So far you have learnt that:

- The term xenobiotic is derived from the Greek words xenos which means foreigner, stranger and Bios means life, tikos means on). A xenobiotic is a chemical substance found within plant or animal that is not naturally produced. It can also cover substances that are present in much higher concentration than are usual.
- The major sources of xenobiotics are food additives such as colourings, flavours, preservatives, etc. The other sources are fungicides, pesticides, herbicides, antibiotic or hormone residues, various other drugs and environmental pollutants such as smoke produced by automobiles, brick-kilns, industries, crackers and fireworks used on festive occasions. Xenobiotic compounds are man-made chemicals that are present in the environment at unnaturally high concentrations.
- The broad categories of Xenobiotic pollutants are Recalcitrant hydrocarbons, haloalkyl propellants and solvents, recalcitrant nitroaromatic compounds, polychlorinated biphenyls, (PCBs) and dioxins, synthetic polymers, alkyl benzyl sulphonates, petroleum hydrocarbons and acid mine drainage.
- There are five possible processes of Xenobiotics transport across the intestine. They are active transport, pinocytosis, filtration through “pores”, lymphatic absorption and passive diffusion.
- Xenobiotics interact with community by various methods such as through patient excretion, direct release into the wastewater system from manufacturing, hospitals or disposed through toilets and sinks, terrestrial depositions, i.e. irrigation with treated and untreated wastewater, sludge, application to land, leaching from solid waste landfills, non-pharmaceutical industrial sources, i.e. plastic products, agricultural wastes such as herbicides, pesticides and fertilizers, through ageing infrastructures, i.e. synthetic compounds such as analgesics and antihistamines which were exposed in streams and rivers, drugs associated with plant health and herbal preparations and their interaction with the environment
- There are various effects of xenobiotics such as nausea, hepatovascular diseases, cardiovascular disease, neurological diseases, endocrine disturbance etc. The

effects on Aquatic Ecosysteminclude poor water quality, reduce population of desirable fishes. The effects on plants include necrosis, low productivity etc.

- There are various bacteria which can stabilize and degrade the xenobiotics in different ecosystems. Species such as Streptomyces, Pseudomonas, Achromobacter, Rhodococcus, Bacillus fusiformis etc. can degrade naphthalene. Pseudomonas species, Massillia species, Sphingobium, Mycobacterium, Brevibacillus, Sphingomonas can degrade polycyclic aromatic hydrocarbon. Certain species of Microbacterium, Martelella, Ochrobactrum, Rhodococcus opacus, Pseudomonas aeruginosa, Pseudomonas citronellolis can degrade Anthracene. Petachlorophenol can be stabilized by the Kocuria species, Comamonas testosteroni, Sphingobium species, Bacillus cereus and Sphingomonas chlorophenolica. Chloroaniline can be degraded by Acinetobacter baylyi, Delftia tsuruhatensis, Acinetobacter baumannii and Pseudomonas putida. 1,2,4-trichlorobenzene can be stablized by Pseudomonas utida and Bordetella spceis. 2-chlorobenzoic acid can be degraded by Pseudomonas, Enterobacter, Acinetobacter and Corynebacterium spceis. Fluoranthene can be stablized by Herbaspirillum chlorophenolicum. Spcies of Klebsiella, Bacillus, Diaphorobacter and Pseudoxanthomonas degrade Pyrene. Many bacteria such as Acgromobacter denitrificans, Arthrobacter, Agrobacterium, Ochrobatrum, Enterobacter, Rhodococcus can stablize xenobiotics (Phthalate) in natural ecosystems.
- The recalcitrant chemicals can resist biodegradation for various reasons and some are more resistant than others. The failure of a compound to be degraded or its slow biodegradation may be because of environmental and chemical factors. Many recalcitrant chemicals are toxic to some extent to some or most microbes. The examples include most phenols (especially chloro- and nitro-derivatives) and fungicides. Concentration is a major factor in the fate of recalcitrant compounds in the environment.
- The recalcitrant xenobiotic compounds can be grouped into the following 6 types namely Halocarbons, Polychlorinated biphenyls, synthetic polymers, alkylbenzyl Sulfonates, oil Mixtures and Other Xenobiotic Compounds

Terminal Questions

1 (a) Fill in the blank spaces with appropriate words.

Environmental contamination by …….is a global phenomenon as a result of human activities resulting from rise in urbanization and……. There are numerous sources of xenobiotics ranging from pharmaceuticals to……. In recent time, the demand for pharmaceuticals versus population growth has placed the public at risk. In addition, the making of unlawful drugs has led to the discharge of harmful carcinogens into the environment. The release of these harmful pollutants results in numerous short- and long-term effects to the environment. Originally, the term xenobiotic comes from the Greek word……, which means ……..or **strange**, and 'bios', which means life. Xenobiotics are chemical compounds exhibiting **abnormal** structural characteristics. The presence of ……drugs in the human body which may not be produced by the body itself nor is a

normal part of diet is example of…….. At times, a natural substance can be defined as a xenobiotic if it found its way into humans or other animals.

2 (a) What do you understand by the word "xenobiotics"? Explain with examples.

(b) Give a note on Sources of Xenobiotics

3 (a) Describe the types of xenobiotics.

(b) Describe about the Transfer of xenobiotics in human body.

4 (a) What are xenobiotics? Describe their stabilization in disturbed ecosystems.

5 (a) Discuss about the recalcitrant compounds

6 (a) Fill the blank spaces with appropriate words.

The ……..chemicals can resist biodegradation for various reasons and some are more …….than others. The failure of a compound to be degraded or its slow biodegradation may be because of ……and chemical factors. Many recalcitrant chemicals are ……..to some extent to some or most microbes. The examples include most ……..and fungicides. Concentration is a major factor in the fate of recalcitrant compounds in the environment. If the concentration is too high, problems of toxicity may……. If a chemical enters an ……with very few organisms in it, then the degradation will either be slow or will not occur. Pristine water in the upper reaches of a stream, ground water (in aquifers), and subsoil have very small microbial communities, thus the recalcitrant compounds entering these environments are degraded slowly. The presence of acclimated organisms, which are previously exposed to a target compound, can greatly accelerate biodegradation. In environment any compound or molecule that persists in nature for long time and resist degradation called…….. ……..

(b) Which is example of Xenobiotic? (Dioxin/Natural Hormone/Amylase enzyme/Vitamin D)

(c) Which is a superbug bacterium? (Pseudomonas putida /Steptococcus/Vibrio cholera/Bacillus)

(d) In word Xenobiotics, Xenos means (Toxic/Polluted/Foreign/Dirty)

(e) What are the types of recalcitrant compounds?

7 (a) Describe the effects of xenobiotics and recalcitrant compounds.

b) How xenobiotics are interacted with community? Explain

c) Describe the process of biodegradation of xenobiotics and recalcitrant compounds.

Answers

1 (a) xenobiotics, industrialization, agriculture, xenos, foreign, antibiotic, xenobiotic

2 (a) see the section 10.2.

(b) See the section 10.2.1

3 (a) See the section 10.2.1

(b) See the section 10.2.2

4 (a) See the section 10.2.5

5 (a) See the section 10.3

6 (a) Recalcitrant, resistant, environmental, toxic, phenols, arise, environment, recalcitrant compounds

(b) Dioxin

(c) *Pseudomonas putida*

(d) Foreign

(e) See the section 10.3 under heading “Types of Recalcitrant compounds”

7 (a) See the section 10.2.4

(b) See the section 10.2.3

(c) See the section 10.2.5 under heading “Degradation of Xenobiotics” and Section 10.3 under heading “General Features of Biodegradation of Recalcitrant compounds”

Unit 11 Genetic Engineering: Basic Concepts

Unit Structure

11.0 Learning Objectives

After studying this unit you will be able to understand:

- What is genetic engineering?
- About selection and cloning of genes.
- About recruitment of GEOs.
- About survival and significance of GMOSs
- What are applications of GEOs
- About gene transfer in aquatic terrestrial and specific ecosystems

11.1 Introduction

As you know that many sciences are useful to human kind and genetic engineering is one of them. Genetic engineering is also called **recombinant DNA technology**. It involves the group of techniques used to cut up and join together genetic material, especially DNA from different biological species. Genetic engineering is literally technology of biological sciences. Many animal and plant have been developed due to genetic engineering. Genetic engineering also produces different types of microbes which can degrade the environmental pollutants. The term 'genetic engineering' stands for human alteration of the genetic code of

an organism, so that its biosynthetic properties are changed. An organism that is generated through genetic engineering is considered to be genetically modified (GM) and the resulting entity is a genetically modified organism (GMO). The first GMO was a bacterium generated by Herbert Boyer and Stanley Cohen in 1973. **Rudolf Jaenisch** created the first GM animal when he inserted foreign DNA into a mouse in 1974. Genetically engineered human insulin was produced in 1978 and insulin-producing bacteria were commercialized in 1982. Genetically modified food has been commercialized since 1994, with the release of the **Flavr Savr tomato**. The Flavr Savr was engineered to have a longer shelf life, but most current GM crops are modified to increase resistance to insects and herbicides. Therefore, genetic engineering is boon to human being. In this unit you will learn about the genetic engineering, selection of cloning and genes, genetic modifies organisms and their applications.

11.2 Genetic Engineering

Genetic engineering, also called **genetic modification** or **genetic manipulation**, is the direct manipulation of an organism's genes using **biotechnology**. It is a set of technologies used to change the genetic makeup of cells, including the transfer of **genes** within and across species boundaries to produce improved or novel organisms. New DNA is obtained by either isolating or copying the genetic material of interest using recombinant DNA methods or by artificially synthesizing the **DNA**. A construct is usually created and used to insert this DNA into the host organism. **Paul Berg is regarded as father of genetic engineering.** The first recombinant DNA molecule was made by **Paul Berg** in 1972 by combining DNA from the monkey virus **SV40** with the lambda virus. As well as inserting genes, the process can be used to remove, or **"knock out"**, genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome.

Genetic engineering has been applied in numerous fields including research, medicine, industrial biotechnology and agriculture. In research GMOs are used to study gene function and expression through loss of function, gain of function, tracking and expression experiments. The same techniques that are used to produce drugs can also have industrial applications such as producing enzymes for laundry detergent, cheeses and other products.

The rise of commercialized GMOs has provided economic benefit to farmers in many countries. Genetic engineering is a process that alters the genetic structure of an organism

by either removing or introducing DNA. Unlike traditional animal and plant breeding, which involves doing multiple crosses and then selecting for the organism with the desired phenotype, genetic engineering takes the gene directly from one organism and inserts it in the other. This is much faster, can be used to insert any genes from any organism and prevents other undesirable genes from also being inserted.

The DNA can be introduced directly into the host organism or into a cell that is then fused or hybridised with the host. This relies on recombinant nucleic acid techniques to form new combinations of heritable genetic material followed by the incorporation of that material either indirectly through a vector system or directly through micro-injection, macro-injection or micro-encapsulation.

Plants, animals or microorganisms that have been changed through genetic engineering are termed genetically modified organisms or GMOs. If genetic material from another species is added to the host, the resulting organism is called **transgenic**. If genetic material from the same species or a species that can naturally breed with the host is used the resulting organism is called **cisgenic**. If genetic engineering is used to remove genetic material from the target organism the resulting organism is termed a knockout organism. The deliberate modification in genetic material of an organism by changing the nucleic acid directly is called **genetic engineering**. Genetic engineering holds the potential to extend the range and power of every parameter of biotechnology.

The techniques will be used widely to improve the existing microbial processes through improving the existing cultures and discarding the unwanted by-products. Of course, during this decade the rDNA technology will establish fully the basis of new microorganisms with new metabolic properties.

11.3 Selection and Cloning Of Genes

Selection of genes: In some cases the accuracy of selection for a gene can be measured using calibrated tool or a scale. Measurements of a gene can be replicated with high reliability. Alternatively some genes are difficult to measure on an objective scale, in which case a well-designed subjective scoring method can be effective. Because close relatives share many genes, an examination of the relatives of a candidate for breeding can improve accuracy of selection. The more complete the genealogical record and pedigree are the

most useful for the selection of genes. A pedigree in most useful when the heritability of a trait is relatively low, especially for traits that are expressed later in life or in only one gender (male or female). Reproductive technique can be used to enhance the rate of genetic progress. These reproductive technologies reduce the generation intervals and increase selection intensity.

Cloning of genes: There are various techniques of cloning of genes which are described below:

A. Cloning in Prokaryotes:

1) Isolation of DNA to be cloned: The DNA of interest also called **target DNA** or **genomic DNA** or **complementary DNA** or **synthetic DNA**. The genomic DNA of interest if contained in a particular restriction fragment that can be isolated from gel after electrophoresis. Otherwise, a complementary DNA (cDNA) fragment is prepared directly by using mRNA as template. The polyadenylated mRNAs are separated from other types of RNAs through affinity column chromatography. These mRNAs are then copied to cDNAs with the help of reverse transcriptase. In these cases as the cDNA is obtained from mRNA, so it must contain the uninterrupted coding sequence of gene and the recombinant DNA molecule will synthesize the eukaryotic gene product in prokaryotic cell. One can also synthesize the desired DNA fragment by machine.

2) Insertion of Foreign DNA Fragment into a Vector: The cDNA thus isolated above or obtained from gene bank is fragmented by using the specific restriction enzyme to develop specific cohesive ends. The cloning vector is also treated with the same restriction enzyme, so that the cohesive ends are generated. For insertion of double stranded cDNA into a cloning vector, it is necessary to add to both termini single stranded DNA sequence which should be complementary to a tract of DNA at the termini of linearized vector. In order to get efficient formation of recombinant DNA molecules, addition of sticky ends on both termini is necessary.

3) Transfer of Recombinant DNA into Bacterial Cell: Before the recombinant DNA can be bulked up by cloning, it must be taken up by a suitable bacterial host cell, which is then said to be transformed, i.e., a host bacterial cell must accept the plasmid with the foreign gene, get it incorporated into its genome and start transcribing that gene. The event of

entering the plasmid with foreign DNA into the cell is known as **"transformation"**. A mild heat shock is given to the mixture which results in the uptake at higher frequency of the DNA. The selection of transformed cells is done by allowing the bacteria to grow in antibiotic selection medium.

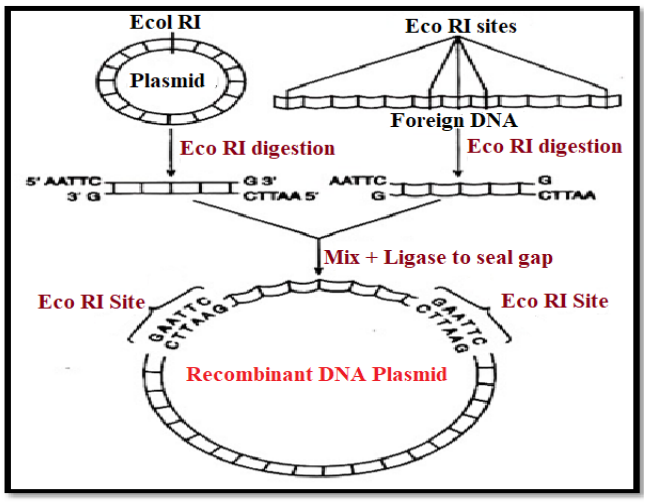


Fig 1: Construction of recombinant DNA plasmid through the use of the restriction enzyme ECO-RI. The Eco-RI cuts both the plasmid and the foreign DNA by mixing

B. Cloning in Eukaryotes

In eukaryotes the nucleus is separated from the rest of cell through nuclear membrane, many of the genes are split genes with exons and introns. As such genetic engineering with eukaryotes needs special methods. When eukaryotic genes are cloned in prokaryotes, the split genes cannot be correctly expressed, because prokaryotes do not have the machinery for splicing out the RNA transcribed from the introns of a gene. Therefore, the eukaryotic cells are needed for cloning and expression of cloned eukaryotic genes.

Among eukaryotes, DNA cloning has been done in yeast, mouse and in higher plant species. In yeast, a 2μ plasmid DNA is an appropriate cloning vehicle, which can be transferred through efficient transformation method. This involves protoplast production followed by PEG directed introduction of DNA into protoplasts.

Detection of Recombinant Clone: From the large number of colonies produced by transformation to select or screen out the few colonies which contain the recombinant plasmid — the use of antibiotics is one of the most easy and useful methods for this purpose. The transformed cells can be plated on selection medium containing different antibiotics. The colonies which grow, can be said to have a plasmid, as the antibiotic resistance gene of plasmid enables the bacteria to grow. For example, the plasmid pBR 322 contains genes for ampicillin resistance (amp^r) and tetracycline resistance (tet^r). Thus the trans-formants

can be detected by their plating potential on medium containing either (or both) of these antibiotics.

11.4 Recruitment of GEOs (GMOs)

Genetically Modified **Organisms** (GMOs) include plants, **animals** and microbes which have been engineered in the **laboratory**. In conventional livestock production, crop farming, and even pet breeding, it has long been the practice to breed select individuals of a species in order to produce offspring that have desirable traits. In genetic modification, however, recombinant genetic technologies are employed to produce organisms whose genomes have been precisely **altered** at the molecular level, usually by the inclusion of genes from **unrelated species** of organisms that code for **traits** that would not be obtained easily through conventional selective breeding. It must isolate the gene they want to insert into the **host organism** and combine it with other genetic elements, including a promoter and terminator region and often a selectable marker. A number of techniques are available for inserting the isolated gene into the host genome. Recent advancements using genome editing techniques, notably **CRISPR**, have made the production of GMO's much simpler. **Herbert Boyer** and **Stanley Cohen** made the first genetically modified organism in **1973**, bacteria resistant to the antibiotic **kanamycin**. The first genetically modified animal, **a mouse**, which was developed by **Rudolf Jaenisch** in the year **1974**. In 1994 the **Flavr Savr tomato** the first commercialized genetically modified food.

Bacteria are the simplest organisms to engineer and have been used for research, food production, industrial protein purification, agriculture, bioremediation etc. There is potential to use them for environmental purification or as medicine. Fungi have been engineered with much the same goals. Viruses play an important role as vectors for inserting gene into host. This use is especially relevant to human gene therapy. There are proposals to remove the virulent genes from viruses to create vaccines. Plants have been engineered for scientific research, to create new colors in plants, deliver vaccines and improved crops. The majority of GEOs are engineered for herbicide tolerance or insect resistance. Golden rice has been engineered with three genes that increase its nutritional value. GM crops are as bioreactors for the production of **biopharmaceuticals, biofuels** or **medicines**.

Table-1: Showing important GEOs (GMOs) and their applications

Name of GMO	Application
Microorganisms	
Aspergillus	Food fermentations
Escherichia coli	Therapeutic protein production
Streptomyces	Antibiotics, antitumor
Bacillus	Industrial enzymes, Fine chemicals, Antibiotics, Insecticides
Plants	
Cotton	Herbicide tolerant , Insect tolerant
Ornamental Flowers	Altered colour , Longer Shelf life
Maize	Herbicide tolerant , Insect tolerant
Oil Seed rape	Herbicide tolerant
Potato	Increase Starch Content
Soybean	Herbicide tolerant
Sugarbeet	Herbicide tolerant
Golden Rice	Enhance Vitamin A
Papaya	Virus Resistance
Animals	
Genetically Modified Salmon Fish	High Production of fish
Genetically Modified Pig (ENVIROPIG)	Less phosphorus consumed by the pig.
Genetically Modified Mosquitoes	Control Disease in Human

Fauna are generally much harder to transform as compared to plants. The massive majority of animals or fauna are still at the research stage. Mammals are the best model organisms for humans, making ones genetically engineered to resemble serious human diseases important to the discovery and development of treatments. Human proteins expressed in mammals are more likely to be similar to their natural counterparts than those expressed in plants or microorganisms. Livestock are modified with the intention of improving economically important traits such as growth-rate, quality of meat, milk composition, disease resistance and survival. Genetically modified fish are used for scientific research, as pets and as a food source. Genetic engineering has been proposed as a method to control population of insects specially mosquitoes, a vector for many deadly diseases.

As only a single cell is transformed with genetic material, the organism must be regenerated from that single cell. In plants this is accomplished through tissue culture. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. Further testing using PCR, Southern hybridization, and DNA sequencing is conducted to confirm that an organism contains the new desirable genes.

Gene targeting techniques, which create double-stranded breaks and takes advantage on the cells natural homologous recombination repair systems, have been developed to target insertion to exact locations. Genome editing uses artificially engineered nucleases that create breaks at specific points. There are four families of engineered nucleases: **meganucleases, zinc finger nucleases, transcription activator-like effector nucleases. etc**. **Transcription activator-like effector** (TALENs) has greater target specificity, while CRISPR is easier to design and more efficient.

11.5 Survival and Significance of Geos (GMOs)

Survival of GEOs is one of the major issues for biotechnologists. As you know that Genetically Engineered Organisms or Genetically Modified Organisms are those plants and animals which are produced by genetic engineer. These organisms can survive in extreme environmental conditions. These can survive in drought, low or high temperature, xeric condition. The survivorship of these organisms totally depends on the genes. Genes make them adapted to different environmental conditions. The development of gene transfer system together with combined “omics” platforms has facilitated the understanding of physiology and biochemistry of plants adaptive responses to unfavourable conditions. Gene transfer responses include profound morphological, physiological and metabolic changes which depend on the induction and repression of numerous genes and allow plants to survive and reproduce under unfavourable condition.

Bacteria were the first organisms to be genetically modified in the laboratory, due to the relative ease of modifying their chromosomes. This ease made them important tools for the creation of other GMOs. Genes and other genetic information from a wide range of organisms can be added to a plasmid and inserted into bacteria for storage and modification. Bacteria are cheap, easy to grow, clonal, multiply quickly and can be stored at −80 °C . Once a gene is isolated it can be stored inside the bacteria. A large number of custom plasmids make manipulating DNA extracted from bacteria relatively easy.

The simplest model organisms come from bacteria. Scientists can easily manipulate and combine genes within the bacteria to create novel or disrupted proteins and observe the effect this has on various molecular systems. Researchers have combined the genes from bacteria and archaea, leading to insights on how these two diverged in the past.

For over a century bacteria have been used in agriculture. Crops have been inoculated with Rhizobia to increase their production or to allow them to be grown outside their original habitat. Survival of bacteria in this environment is easy. Application of *Bacillus thuringiensis* (Bt) and other bacteria can help protect crops from insect infestation and plant diseases. Markers have also been added to aid in tracing the spread of the bacteria. The bacteria that naturally colonize certain crops have also been modified, in some cases to express the Bt genes responsible for pest resistance. *Pseudomonas* strains of bacteria cause frost damage by nucleating water into ice crystals around themselves.

Most vaccines consist of viruses that have been attenuated, disabled, weakened or killed in some way so that their virulent properties are no longer effective. Genetic engineering could theoretically be used to create viruses with the virulent genes removed. This does not affect the viruses infectivity, invokes a natural immune response and there is no chance that they will regain their virulence function, which can occur with some other vaccines. As such they are generally considered safer and more efficient than conventional vaccines, although concerns remain over non-target infection, potential side effects and horizontal gene transfer to other viruses. Another potential approach is to use vectors to create novel vaccines for diseases that have no vaccines available or the vaccines that do not work effectively, such as AIDS, malaria, and tuberculosis. The most effective vaccine against Tuberculosis, the Bacillus Calmette–Guérin (BCG) vaccine, only provides partial protection.

Plants have been engineered for scientific research, to display new flower colors, deliver vaccines and to create enhanced crops. Many plants are pluripotent, meaning that a single cell from a mature plant can be harvested and under the right conditions can develop into a new plant. This ability can be taken advantage of by genetic engineers; by selecting for cells that have been successfully transformed in an adult plant a new plant can then be grown that contains the transgene in every cell through a process known as tissue culture.

Much of the advances in the field of genetic engineering has come from experimentation with tobacco. Major advances in tissue culture and plant cellular mechanisms for a wide range of plants has originated from systems developed in tobacco. It was the first plant to be altered using genetic **ENGINEERING** and is considered a model organism for not only genetic engineering, but a range of other fields. As such the transgenic tools and procedures

are well established making tobacco one of the easiest plants to transform. Another major model organism relevant to genetic engineering is *Arabidopsis thaliana.* Its small genome and short life cycle makes it easy to manipulate and it contains many homologues to important crop species. It was the first plant sequenced, has a host of online resources available and can be transformed by simply dipping a flower in a transformed *Agrobacterium* solution.

There are three main aims to agricultural advancement; increased production, improved conditions for agricultural workers and sustainability. GM crops contribute by improving harvests through reducing insect pressure, increasing nutrient value and tolerating different abiotic stresses. Despite this potential, as of 2018, the commercialized crops are limited mostly to cash crops like cotton, soybean, maize and canola and the vast majority of the introduced traits provide either herbicide tolerance or insect resistance. Geographically though the spread has been uneven, with strong growth in the Americas and parts of Asia and little in Europe and Africa. Its socioeconomic spread has been more even, with approximately 54% of worldwide GM crops grown in developing countries in 2013.

The majority of GM crops have been modified to be resistant to selected herbicides; usually a glyphosate or glufosinate based one. Genetically modified crops engineered to resist herbicides are now more available than conventionally bred resistant varieties; in the USA 93% of soybeans and most of the GM maize grown is glyphosate tolerant. Most currently available genes used to engineer insect resistance come from the *Bacillus thuringiensis* bacterium and code for delta endotoxins. A few use the genes that encode for vegetative insecticidal proteins. The only gene commercially used to provide insect protection that does not originate from *B. thuringiensis* is the Cowpea trypsin inhibitor (CpTI). CpTI was first approved for use cotton in 1999 and is currently undergoing trials in rice. Less than one percent of GM crops contained other traits, which include providing virus resistance, delaying senescence and altering the plants composition. ***Golden rice is the most well known GM crop that is aimed at increasing nutrient value. It has been engineered with three genes that biosynthesise beta-carotene, a precursor of vitamin A, in the edible parts of rice.***

Plants and plant cells have been genetically engineered for production of biopharmaceuticals in bioreactors, a process known as pharming. Work has been done with duckweed *Lemna minor*, the algae *Chlamydomonas reinhardtii* and the moss *Physcomitrella patens*. Biopharmaceuticals produced include cytokines, hormones, antibodies, enzymes and vaccines, most of which are accumulated in the plant seeds. Many drugs also contain natural plant ingredients and the pathways that lead to their production have been genetically altered or transferred to other plant species to produce greater volume. Other options for bioreactors are biopolymers and biofuels. Unlike bacteria, plants can modify the proteins post-translationally, allowing them to make more complex molecules.They also pose less risk of being contaminated. Therapeutics have been cultured in transgenic carrot and tobacco cells, including a drug treatment for Gaucher's disease.

The vast majority of genetically modified animals are at the research stage with the number close to entering the market remaining small. As of 2018 only three genetically modified animals have been approved, all in the USA. A goat and a chicken have been engineered to produce medicines and a salmon that has increased growth. Despite the differences and difficulties in modifying them, the end aims are much the same as for plants. GM animals are created for research purposes, production of industrial or therapeutic products, agricultural uses or improving their health. There is also a market for creating genetically modified pets. The process of genetically engineering mammals is slow, tedious, and expensive. However, new technologies are making genetic modifications easier and more precise. The first transgenic mammals were produced by injecting viral DNA into embryos and then implanting the embryos in females. The embryo would develop and it would be hoped that some of the genetic material would be incorporated into the reproductive cells. Then researchers would have to wait until the animal reached breeding age and then offspring would be screened for presence of the gene in every cell. The development of the CRISPR-Cas9 gene editing system as a cheap and fast way of directly modifying germ cells, effectively halving the amount of time needed to develop genetically modified mammals.

Significance: Significance of GEOs includes bioremediation, where the bacteria are used to convert pollutants into a less toxic form. Genetic engineering can increase the levels of the enzymes used to degrade a toxin or to make the bacteria more stable under environmental conditions. Bio art has also been created using genetically modified bacteria.

Domestic animals are modified with the intention of improving economically important traits such as growth-rate, quality of meat, milk composition, disease resistance and survival. Animals have been engineered to grow faster, be healthier and resist diseases. Modifications have also improved the wool production of sheep and udder health of cows. Goats have been genetically engineered to produce milk with strong spiderweb-like silk proteins in their milk. They could reduce water pollution since they excrete 30 to 70% less phosphorus in manure. Dairy cows have been genetically engineered to produce milk that would be the same as human breast milk.

Gene therapy uses genetically modified viruses to deliver genes which can cure disease in humans. Although gene therapy is still relatively new, it has had some successes. It has been used to treat genetic disorders such as severe combined immunodeficiency, and Leber's congenital amaurosis. Treatments are also being developed for a range of other currently incurable diseases, such as cystic fibrosis, sickle cell anemia, Parkinson's disease, cancer, diabetes, heart disease and muscular dystrophy. Germline gene therapy results in any change being inheritable, which has raised concerns within the scientific community. Genetically modified fish are used for scientific research, as pets and as a food source. Aquaculture is a growing industry, currently providing over half the consumed fish worldwide. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically clear chorion (membranes in the egg), rapidly develop, and the one-cell embryo is easy to see and microinject with transgenic DNA. Zebrafish are model organisms for developmental processes, regeneration, genetics, behaviour, disease mechanisms and toxicity testing.

11.6 Applications Of Geos

There are various applications of GEOs which are summarized in Fig-2 and also discussed in the following text.

As you know genetically modified organism is an organism whose genetic material or sequence has been altered. They are the sources of genetically modified foods. Genetic alteration can occur naturally or artificially. Naturally, genetically modified organisms occur as an impact of other factors apart from human will. For instance, the penetration of a gene into a foreign cell could result to development of a naturally genetically modified organism.

Artificially, GEOs have been developed depending on desired characteristic.

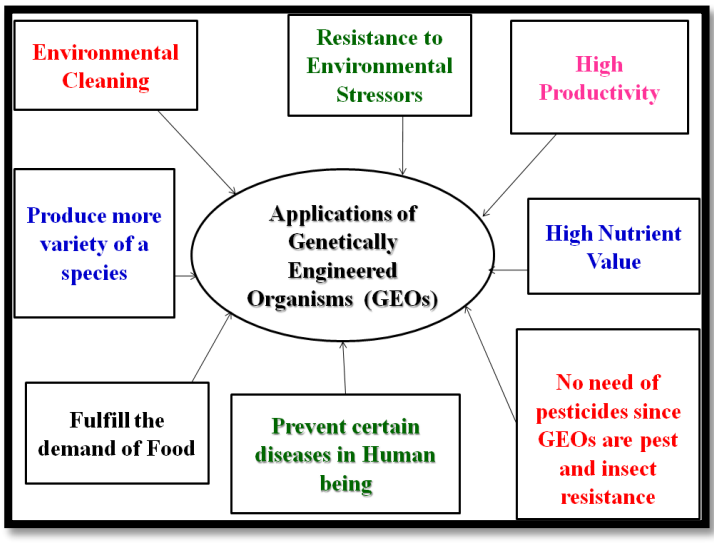


Fig 2: Showing applications of Genetically Engineered Organisms (GEOs)

Numerous genetic engineering techniques have been developed and used in the development of these organisms. These techniques which are based on circumstances which could occur naturally include; deletion, insertion and mutation of specific genes. Some organisms whose genetic makeup has been successfully modified include bacteria, yeasts, plants, fish and mammals. Other than food, GEOs have been used in the production of other commercially products like proteins.

Genetically modified bacteria have numerous benefits. For example there has been production of large amounts of commercially viable human proteins that is used in production of medicine. Insulin, a protein used to treat diabetes has been easily produced by these bacteria. Human growth hormone, used to treat dwarfism has also been produced. Other genetically modified organisms have been used in production of enzymes and clotting factors. For example, a drug Atryn, an anticoagulant has been obtained from the milk of a genetically modified goat.

Organisms that are of medicinal value have been cultivated in bioreactors rather than in the fields. Proper examples include; *Chlamydomonas reinhardtii* which is an alga and *Physcomitrella patens* which is a moss. Cultivating of organisms in bioreactors allows close monitoring so as to provide the required results. Other genetically modified organisms have been used as models in researches that are important in the development and establishment of treatments for different diseases.

In agriculture, crops with better traits like yield, vigor and resistance to pests and diseases have been developed through genetic engineering. A good example is the genetically modified papaya grown in Hawaii. The modified papaya is resistant to the **Ring spot virus** and it also produces the *Bacillus thuringiensis* toxin which is considered not harmful to human but plays a great role as an insecticide. Crops have been engineered to enhance production with longer shelf life. For example, corn and the poplars have been genetically modified and used to produce biofuel which have been used as an efficient substitute of petroleum products. There is development of animals with traits that are desirable compared with their natural counterparts. For example, Yorkshire pigs have been genetically modified to produce a type of pig that digests plant phosphorus. They are capable of producing enzyme phytase in their saliva, which digests phosphorous unlike their counterpart pigs. According to Centre for ecogenetics and Environmental Health "**USA is the largest producer of GMOs, followed by, Brazil, Argentina, India, Canada, China, Paraguay, South Africa, Uruguay and Bolivia".**

Transgenics have been produced in a variety of animal species, e.g., mice (including rats), rabbits, swine, sheep, goat, cattle, poultry, fish, amphibians, insects and nematodes. However, these activities are so far limited to experimental stages, and production technologies based on transgenic animals have not yet reached commercial applications:

Advantages of GMOs: There are various advantages and disadvantages of GMOs which are summarized in Fig-3 and also pointed below:

1. **<u>They allow more profit</u>:** GMOs are an effective way to provide farmers a larger profit, while making them spends less time on resources. GMOs provide better economic value as compared to other non-GMOs. Since GMOs are designed to resist pests, there will be no need for pesticides to be used, which means more savings.
2. **<u>Introduce the knowledge of genetic alterations</u>:** It is another important advantage of GMOs. This is done through mapping genetic material for GM crops. This way, we would get the ability to enhance crop genes and make them more beneficial for human production and consumption. Plants can be engineered to

resist temperature or produce higher yields, which is good for regions where climate limits productivity.

3. **They are known to decrease food prices:** Advanced crops and lower costs can lead to cheaper food. This will certainly help families who cannot afford to buy their needed supply for everyday consumption. Therefore, food supply and availability of food is assured.
4. **Add more nutritional value to crops:** The GMO method can put in added nutritional value to crops that lack necessary vitamins and minerals. Considering that there are places in the world relying on rice or corn as their daily staple, plant genes may be added to these crops to increase their nutritional value. Therefore, malnourished populations receive more nutrients from diet. For example Golden rice which has more vitamin A.
5. **Products are found to be safe:** The precise evaluation and testing of GMOs crops and other products means they are safe for human consumption. In fact, research shows that they are safer compared with traditional crops.

Disadvantages:

1. **Ecological imbalance:** GMOs are believed to be dangerous to some insects because new crop genes can be deadly to them. This is worth noting when it comes to certain insects, such as butterflies, that are not actually dangerous to agricultural ecology.
2. **Changing the field of agriculture:** The process of making GMOs includes adding new genetic material into an organism. In agricultural ecology, this means introducing new genes in the genome of crops like corn. Research on the effects of cultivation of GM crops in a large scale has sparked various concerns, specifically those ideas on ecosystems with GMO strains. As proven by certain studies, GMO strains have the potential to change agriculture.
3. **They can damage the environment:** Genetically modified organisms specially crops can cause a threat to the environment due to the fact that they are not a natural way to plant and cultivate plants.

4. **They cause unwanted residual effects:** A genetically modified organism can leave unwanted residual substances that can remain in the soil for extended periods

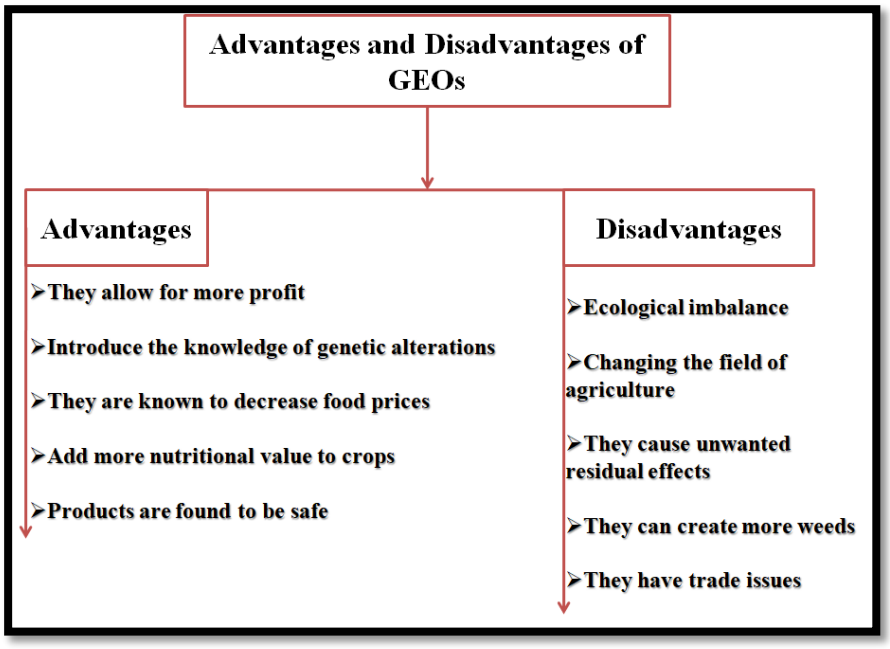


Fig-3: Showing advantages and disadvantages of Genetically Engineered Organisms (GEOs)

of time. Agricultural regulators were alerted by research that strains from GM crops would remain in the soil for years after the crops were removed. Scientists even reported that despite the absence of these plants, the strain persisted for up to more than 5 years.

5. **They can create more weeds:** Take note that engineered crops can act as mediators in transferring genes to wild plants, which can create more weeds. To keep these new weeds under control, scientists then invented new herbicides that were not necessary for non-GMO weeds. These chemicals are also toxic to various mammals and amphibians, who are feeding on GMO crops. Researcher also observed that the uptake of these herbicides also has toxic consequences on aquatic ecosystems.

6. **May be threatened to crop diversity:** There is opposition to introducing GM genes on genetic diversity because these genes can spread to other organic farm crops and threaten crop diversity in agriculture. And if crop diversity decreases, it will have a direct impact on our entire ecosystem and would affect the population dynamics

of other plants and animals. However, when a large scale plantation releases a GM strain during pollination, this risk increases, where the cross pollination to non-GM plants could create a hybrid strain. This means there is a greater possibility of ecological novelty or new artificial strains that are being introduced into the environment that could potentially reduce biodiversity through competition.

7. **They have trade issues:** In many countries, there may be problems regarding trade matters, such as tariff and quota.

11.7 Gene Transfer in Aquatic, Terrestrial and Specific Ecosystems

Ecosystems are important potential sites for gene transfer between indigenous bacteria and released genetically engineered microorganisms. Legislation governing GEM releases, and other practical considerations, have resulted in microcosms, of varying complexity, being used to study gene transfer in ecosystems. At present, little is known about gene transfer between microorganisms in the ecosystems, its frequency, and what impact this may have on how microbial populations adapt to changing environment. The increasing likelihood of releasing genetically engineered microorganisms into the ecosystems has emphasized this lack of knowledge and, as a result, concerns have been expressed that recombinant DNA could be transferred to the indigenous community with undesirable consequences, such as the disruption of microbial population dynamics. Hence, it is becoming increasingly important to understand gene transfer in the various ecosystems. For gene transfer in aquatic ecosystem we should kept following points in mind.

- pH of water should be according to the microbes which are being transferred in aquatic ecosystem.
- Temperature and other important parameters such as dissolved oxygen, biological oxygen demand, transparency, turbidity etc. should be monitored and adjusted according to microbial strains which are being inserted in aquatic ecosystem.
- Aerobic microbes should be allowed if there is dissolved oxygen in aquatic ecosystem; if there is no dissolved oxygen we should insert anaerobic microbes.
- Different pollutants of aquatic ecosystem such as mercury, cadmium, arsenic, chromium etc should be analyzed.

- Microcosm should be prepared according to aquatic environment.
- If we have to degrade the environmental pollutants, for this purpose we should use appropriate genetically modified organisms to degrade these pollutants.

Although gene transfer is now thought to occur throughout the biosphere, nutrient-rich sites have attracted particular attention. Such sites support large population densities of metabolically active microorganisms which, in vitro studies have shown, can result in high gene transfer-frequencies. Aquatic environments contain many nutrient-rich habitats. Sediments, often rich in organic material, support bacterial populations. Nutritionally rich, complex, active communities can also be found in river epilithon and other analogous biofilms which form at solid/water interfaces. Epilithic bacteria are embedded within a polysaccharide matrix which both protects the bacteria and adsorbs dissolved and particulate organic matter from the overlying water. Similar biofilms can be found in percolating filter-beds, the second most common sewage-treatment process world-wide. Filter-beds, like other treatment facilities, are nutrient-rich environments supporting high population densities of metabolically active bacteria. They are also potentially the first destinations where many released GEMs can mix with an environmental bacterial community.

For example **Bale** and **Hill** investigated gene transfer in river epilithon. This consisted of a 500 ml beaker containing 100–300 ml of a liquid medium. They used membrane filter of 25–47 mm diameter, 0.22–0.45-μm pore, inoculated with a suitable donor and recipient. This was attached to a stone with rubber bands, which was submerged in the liquid medium. Mating then occurred during a 24 h incubation. The liquid medium, filters and underlying stone-surfaces were then sampled and the numbers of donors, recipients and transconjugants enumerated using standard plate-counting techniques.

There are various techniques of gene transfer in terrestrial ecosystems. Terrestrial ecosystem mainly polluted by the dumping of solid waste and excessive use of agro-chemicals (insecticides, pesticides, molluscides, fungicides, rodenticides, synthetic fertilizers etc). Various organisms specially microbes are used to remove contamination from the land. genetically engineered microbes can degrade land pollutants at very fast rate. For gene transfer we should consider following important points:

- Although genetically modified microbes are adapted to harsh conditions, however, pH of the soil should be according to genetically modified microbes.
- Temperature and other parameters of the soil should be monitored.
- Water holding capacity, moisture content, organic matter and microbial communities of the soil should be analyzed.
- Microcosm should be developed according to terrestrial ecosystem.

Summary

In this unit we have discussed various aspects of genetic engineering, GMOs and Gene transfer. So far you have learnt that:

- Genetic engineering is also called recombinant DNA technology. It involves the group of techniques used to cut up and join together genetic material, especially DNA from different biological species.
- New DNA is obtained by either isolating or copying the genetic material of interest using recombinant DNA methods or by artificially synthesizing the DNA. A construct is usually created and used to insert this DNA into the host organism. Paul Berg is regarded as father of genetic engineering. The first recombinant DNA molecule was made by Paul Berg in 1972 by combining DNA from the monkey virus SV40 with the lambda virus. As well as inserting genes, the process can be used to remove, or "knock out", genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome.
- There are various techniques of cloning of genes. steps of cloning of genes include isolation of DNA to be cloned, insertion of Foreign DNA Fragment into a Vector, transfer of Recombinant DNA into Bacterial Cell
- Genetically Modified Organisms (GMOs) include plants, animals and microbes which have been engineered in the laboratory. Herbert Boyer and Stanley Cohen made the first genetically modified organism in 1973, bacteria resistant to the antibiotic kanamycin. The first genetically modified animal, a mouse, which was

developed by Rudolf Jaenisch in the year 1974. In 1994 the Flavr Savr tomato the first commercialized genetically modified food.

- Fauna are generally much harder to transform as compared to plants. The massive majority of animals or fauna are still at the research stage. Mammals are the best model organisms for humans, making ones genetically engineered to resemble serious human diseases important to the discovery and development of treatments. Human proteins expressed in mammals are more likely to be similar to their natural counterparts than those expressed in plants or microorganisms.
- Survival of GEOs is one of the major issues for biotechnologists.
- These organisms can survive in extreme environmental conditions. These can survive in drought, low or high temperature, xeric condition. The survivorship of these organisms totally depends on the genes. Genes make them adapted to different environmental conditions. The development of gene transfer system together with combined “omics” platforms has facilitated the understanding of physiology and biochemistry of plants adaptive responses to unfavourable conditions.
- Plants have been engineered for scientific research, to display new flower colors, deliver vaccines and to create enhanced crops.
- Golden rice is the most well known GM crop that is aimed at increasing nutrient value. It has been engineered with three genes that biosynthesise beta-carotene, a precursor of vitamin A, in the edible parts of rice.
- Genetically modified fish are used for scientific research, as pets and as a food source. Aquaculture is a growing industry, currently providing over half the consumed fish worldwide. Through genetic engineering it is possible to increase growth rates, reduce food intake, remove allergenic properties, increase cold tolerance and provide disease resistance. Fish can also be used to detect aquatic pollution or function as bioreactors.

- There are various applications of GEOs which are Environmental cleraning, resistance to environmental stressors, high productivity, produced more variety, fulfill the demand of food, prevent certain diseases in human being, high nutrient etc
- There are few disadvantages of GMOs which are ecosystem imbalance, spark concerns on changing the field of agriculture, can damage the environment, cause unwanted residual effects, threaten crop diversity and have trade issues.
- Ecosystems are important potential sites for gene transfer between indigenous bacteria and released genetically engineered microorganisms.

Terminal Questions

1 (a) Fill in the blank spaces with appropriate words.

Genetic engineering, also called ……..or……., is the direct manipulation of an organism's genes using…….. It is a set of technologies used to change the genetic makeup of cells, including the transfer of ……..within and across species boundaries to produce improved or novel organisms. New DNA is obtained by either isolating or copying the genetic material of interest using recombinant DNA methods or by artificially synthesizing the…….. A construct is usually created and used to insert this DNA into the host organism. …….is regarded as father of genetic engineering. The first recombinant DNA molecule was made by **Paul Berg** in 1972 by combining DNA from the monkey virus …….with the lambda virus. As well as inserting genes, the process can be used to remove, or ……..genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome.

2 (a) Write a short note on genetic engineering.

(b) What do you understand by GMOs? Describe the GMOs with suitable examples.

3 (a) Describe selection and cloning of genes.

(b) Describe the advantages and disadvantages of GMOs

4 (a) What are the applications of GMOs

5 (a) Discuss the survival and significance of GMOs

6 (a) Fill the blank spaces with appropriate words.

Genetically Modified …….(GMOs) include plants, ……and microbes which have been engineered in the **……..** In conventional livestock production, crop farming, and even pet breeding, it has long been the practice to breed select individuals of a species in order to produce offspring that have desirable traits. In genetic modification, however, recombinant genetic technologies are employed to produce organisms whose genomes have been precisely ……at the molecular level, usually by the inclusion of genes from ……. ……..of organisms that code for ……that would not be obtained easily through conventional selective breeding. It must isolate the gene they want to insert into the …… ……and combine it with other genetic elements, including a promoter and terminator region and often a selectable marker. A number of techniques are available for inserting the isolated gene into the host genome. Recent advancements using genome editing techniques, notably……, have made the production of GMO's much simpler. ……and ……..made the first genetically modified organism in……, bacteria resistant to the antibiotic…….. The first genetically modified animal, a mouse, which was developed by ……in the year 1974. In 1994 the ……the first commercialized genetically modified food.

6 (b) Who is regarded as father of genetic engineering? (Paul Berg/Lederberg/E.P. Odum/Mendel)

6 (c) Advantage of GMOs is/are (Disease resistance/Weather Resistance/More profitable /All of the above)

6 (d) Golden rice is GMC which has more (Vitamin A/Vitamin B/Vitamin D/Vitamin C)

7 (a) Describe the gene transfer in aquatic, terrestrial and specific ecosystems

Answers

1 (a) genetic modification, genetic manipulation, biotechnology, genes, DNA, Paul Berg, SV40, knock out

2 (a) see section 11.2; (b) See section 11.6

3 (a) See section 11.3; (b) See section 11.6 under heading advantages and disadvantages of GEOs

4 (a) See section 11.6 including Figure-2

5 (a) See the section 11.5

6 (a) Organisms, animals, laboratory, altered, unrelated species, traits, host organism, CRISPR, Herbert Boyer, Stanley Cohen, 1973, kanamycin, Rudolf Jaenisch, Flavr Savr tomato

6 (b) Paul Berg

6 (c) All of the above

6 (d) Vitamin A

7 (a) See the section 11.7

Unit 12 The Microcosm

Unit Structure

12.0 Learning Objectives

After studying this unit you will be able to understand:

- What is microcosm?
- What are types of microcosm?
- Role of microcosm?
- What are the factors which affect microcosm?
- About development of strains
- About Batch and continuous culture

12.1 Introduction

As you know microbes play an important role not only in biogeochemical cycles and ecosystem energy flow but also in maintaining ecosystem environmental quality. Literally, natural microbial communities harbour an interesting physiological versatility and catabolic potential for the breakdown of an enormous number of organic molecules, including synthetic compounds. This is because that they can survive in different environmental conditions. They are able to colonize contaminated sites and metabolize some recalcitrant

xenobiotics. Microbial communities represent an important key to understanding the impacts of environmental and anthropogenic factors on ecosystems. Pollution may influence Air, water and soil quality, but little is known on the effects on microbial communities, and consequent impacts on its functioning. Miniaturized ecosystems (**microcosms**) provide the advantage of allowing researchers to investigate under controlled conditions the effects of selective pressures, such as xenobiotic occurrence, on natural microbial communities.

Ecotoxicological effects on microorganisms in water and soil are assessed with standard acute and chronic ecotoxicologic tests on freshwater, marine and soil test organisms. Due to their everywhere distribution in the natural environment, microbial communities are valuable indicators of the occurrence of disturbances due to exogenous physico-chemical parameters. The assessment of variations in microbial community structure is of basic importance in order to evaluate the impact of an environmental stressor. Complex microbial communities may serve as ideal and ecologically relevant toxicity indicators. A number of microbiologically driven processes have been proposed to evaluate the effects of xenobiotics on ecosystems. Moreover, biodegradation is an important but poorly studied and understood fate process that is central to all mitigation strategies when chemicals are detected in the environment. Little is known about the natural variation in biodegradation potential of different environmental compartments or how bacterial presence diversity influences such variation. In this unit we will discuss microcosm designing, uses and role of microcosm.

12.2 The Microcosm

The perfect definition of microcosm is "*Microcosm* means a **miniaturized model** of a natural ecosystem" Small-scale experimental systems also called **microcosms**. According to **Carbonell and Tarazona** (2014) they have historically informed understanding of ecological processes that are applicable at larger scales. Indeed, more recently, the use of **microcosms** has had a role in exploring the consequences of seemingly insurmountable environmental issues, such as the effect of climate change on species **distributions** and food-web **structure**, the effects of biodiversity loss on ecosystem functioning and the impacts of pollution and fisheries on ecosystems.

Microcosms are basically small **ecosystems** in containers. There is a high range of microcosm studies from simple experimentally sown mixtures of two species of plants to sophisticated controlled environments housing entire terrestrial ecosystems such as the **Ecotron facility** at **Silwood Park**. The main role of **microcosms** is that they act as a bridge between theory and **nature**. They do not mirror nature but they can increase our understanding of natural processes by simplifying the **complexities** of our natural environment. Microcosm experiments have played an important role in developing and testing ecological theories and investigating current global change scenarios. According to **Fraser** (2004) many disciplines have utilized microcosms as a powerful research tool especially **microbiologists**, eco-toxicologists, **limnologists** and soil ecologists.

12.3 Microcosm methods

There are various methods of microcosm which are described below:

ASTM Method E 1197-87: This method involves the use of 13.6-1, intact soil cores taken from either natural grassland or from agriculture fields, depending on the application. The biota in the cores is as samples, although crop plants may be planted. The cores are filter with leachate collection funnels are placed in insulated carts to facilitate handling in the greenhouse or growth room. Much of the watering and plant cultural specification follow practices in field agronomy. Measurement of the degradation and leaching rates of contaminants, and the concentration in the soil profile and the plant, were intended as the key endpoints. Plants yield is the only toxicological endpoint usually used, but soil meso-fauna and microbes could also be assessed.

2. NATEC Test System: Kuehle decribed the NATEC test system in 1988, which is large lysimeter containing an undisturbed soil core 72 cm diameter by 43cm deep. The walls of the lysimeter are insulated and the temperature regulated with the heating and cooling coils. Leachates are collected and lysimeter is capped with a quartz dome to allow regulation and sampling of the atmosphere.

3. Litter Microcosms: This microcosm described by **Van Wensem (1989)** is a typical of several designs for investing effects on the processes of litter decomposition. The container used by Van Wensem was 340-cm^3 cylinder with the bottom filled with moist purified sand. An air inlet was provided through the sand, and a lid could be sealed over the top.

Respiration was measured twice a week by analyzing the airstream passing through the litter using an infrared gas analyzer. Litter was sieved and sterilized, rewetted and inoculated with a spore suspension from a fungal culture. After three week, isopods namely ***P. scaber*** collected from the field were sorted for sex and weight and were added. Apart from the respiration measurements, survival, growth and degree of comminution could be easily measured. **Van Wensem (1992)** used this microcosm in an investigation with metal toxicity and found the comminution or fragmentation of the litter was a sensitive endpoint best investigated using a microcosm method.

Chaney et al. (1978) described a larger microcosm for investigation of litter decomposition and it included some mineral soil. With a customized device, they collected undisturbed 16X27cm slabs of litter plus mineral soil from a forest floor, with each microcosm containing 0.5 to 0.9 kg material. After incubation in the dark for 6 months, the microcosms were connected to a gas flow manifold that allowed respiration measurements to be made successively on ten separate microcosms. The microcosms were either collected from contaminated sites or were artificially spiked. This step may have led to effective cross-inoculation among the microcosms, as well as achieved the desired stabilization.

4. Sheppard and Evenden : **Sheppard and Evenden (1994)** presented a variant of the microcosms concept. They used a large 144-1 microcosm as a test environment. This microcosm contained forest litter and soil, and was planted with the grass to provide continuous biological energy inputs. There were plastic lined slots in the microcosm and these were used to insert the test soil. The test soils were in mesh covered petri dishes and were contaminated with the series of concentrations. the test soil was also amended with ground alfalfa which, when moulded, served as a bait to attract micro-arthropods. After 11 days, the petri dishes were removed and placed on Tullgren-type high gradient funnels to extract the micro-arthropods. Only total counts of micro-arthropods were used as the endpoint, although more detailed taxonomic classification is possible. The total counts were interpreted as an ecological indicator resulting from several possible mechanisms. The contaminant may have affected fungal growth, it may have caused avoidance of the test soil by mesofauna, or it may have been related to lethal or sub-lethal effects on the micro-arthropods counted or their prey and predators. The overall test was designed to have the simplicity in use and interpretation often sought in single species tests.

12.4 Role of Microcosm

There are various roles of microcosms which are summarized below:

1) In aquatic ecosystem analysis

2) In soil testing

3) In Toxicology and pollution

4)In vermi-composting

5) In biodiversity analysis

6)In water pollution abatement

12.5 Factors affecting Microcosm

There are several factors which affect the microcosm; some of the important factors which affect microcosm are described below:

1. Temperature: Temperature is the most important factor which determines the rate of growth, multiplication, survival and death of microorganisms in microcosm (miniature of ecosystem). High temperature may damage microbes by denaturing enzymes, transport carriers and other proteins. At very low temperature the microcosms cannot perform properly. There are three categories of the temperature viz. minimum growth temperature, optimum growth temperature and maximum growth temperature. The lowest temperature at which organisms grow is the minimum growth temperature. The temperature at which the most rapid rate of multiplication occur called optimum growth temperature. The highest temperature at which growth occur called maximum growth temperature.

2. pH: As you know pH is negative logarithm of hydrogen ion concentration. The growth in microcosm is greatly affected by the pH of medium in which they are living. Low or high pH may damage the plasma membrane of the microcosmal community. Low or high pH also inhibits the activity of the enzymes.

3. Oxygen: Microbes in microcosm community required for oxygen. Many microbes are aerobic and required oxygen for their survival. On the other hand some microbes do not required oxygen called anaerobic microbes.

4. Energy source: This factor is also responsible for microcosm community. Phototrophs use radiant energy (light) as their primary energy source. In microcosm community chemotrophs use the use the oxidation and reduction of chemical compounds as their primary energy source. Certain microbial communities also use sulfur, phosphorus, potassium, magnesium, calcium, iron and trace elements for their energy requirements.

12.6 Development of Strains and community

Strain: A strain is a group of species with one or more characteristics that differentiate it from other sub group of the same species of the strain. Each strain is identified by the name, number or letter. For Example- ***E. coli*** strain K12. The science and technology of manipulating microbial strains in order to enhance their metabolic capacities for biotechnological applications are referred to as strains development. The main objectives of the strain development are as: rapid growth, genetic stability, non-toxicity to humans, increase productivity, pollution control etc.

Approaches for strain improvement: There are various approaches for strain development such as mutant selection, recombination and recombinant DNA technology. These approaches are described below:

1. Mutant selection: As you know **mutation** is a sudden and heritable change in the genes of an organism. The application of the **mutagens** to induce a mutation is called as **mutagenesis**. The agent which is capable to inducing mutation is called as **mutagens**. There are various chemical mutagens such as **Alkylating** agents, **Acridine** dyes etc. When Mutation occurs without any specific treatment is called **"Spontaneous mutation"** and mutation is resulting due to a treatment with certain agents is known as **"Induced mutation".** A change in the different species due to induced mutation is important aspect. Many mutations bring about marked changes in the biochemical characters of practical interest these are called **major mutation**. These can be used in strain development. For example *Streptomyces griseus*-Streptomycin-Mannosidostreptomycin, *Streptomyces aurofaciens* (S-604) - Produced 6-demethyl tetracycline in place of **Tetracycline**.

Isolation of mutants: There are four methods to detect and isolate mutants. These methods are described below:

a) Replica plating technique: This technique was developed by Lederberg and Lederberg in 1952. This technique is used to detect auxotrophic mutants which differentiate between mutants and wild type strains on the basis of ability to grow in the absence of an amino acid.

b) Resistance selection method: It is the other approach for isolation of mutants. Generally the wild type cells are not resistant either to antibiotics or bacteriophages. Therefore, it is possible to grow the bacterium in the presence of the agent (antibiotics or bacteriophages) and look for survivors.

c) Substrate Utilization method: This method is employed in the selection of bacteria. Several bacteria utilize only a few primary carbon sources. The cultures are plated onto medium containing an alternate carbon sources. Any colony that grows on medium can use the substrate and are possibly mutants and these can be isolated

d) Carcinogenicity test: This test is to identify the environmental carcinogens that cause mutation and induce cancer in organisms. It's based on detecting potential of carcinogens and testing for mutagenecity in bacteria. Ames (1973) developed a method for detection of mutagenicity of carcinogens which is commonly known as Ames test.

1) Recombination: It is defined as formation of new gene combination among those present in different strains. It is used for both genetic analysis as well as strain development. Recombination may be based on transformation, conjugation and transduction.

2) Recombinant DNA Technology: rDNA technology or genetic engineering involves the isolation and cloning of genes of interest, production of necessary genes constructs using appropriate enzymes and then transfer and expression of these genes into a suitable host organism. The recombinant DNA technology has been used to achieve two broad objectives and these objectives are (1) production of recombinant proteins and (2) metabolic engineering. In former, proteins produced by transferred genes/transgene. These are of commercial value. The examples of the recombinant proteins are insulin, interferons etc. In later when metabolic activities of an organism are modified by introducing into it transgene, which affect enzymatic, transport or regulatory function of its cell its known as metabolic engineering. The examples of metabolic engineering are over production of amino acid Isoleucine in *Corynebacterium glutamicum* and ethanol by *E. coli*.

12.7 Designing of Microcosm

The design of microcosm is various types in this section we will discuss the microcosm design in respect to aquatic ecosystem analysis.

(i) Microcosm design. Temperature dependent survival experiments are conducted by using a static renewal system. In this design water samples are collected every 48 h and allowed to acclimatize to the appropriate temperature. The acclimated lake water then added to 1- or 5-gallon aquarium tanks and placed on magnetic stirrers to allow for continuous mixing and circulation of the test water. The lake water in the tanks generally replaced every 24 h.

All experiments should be carried out in either a constant temperature room or an environmental chamber at a constant temperature (15, 25, or 30°C) and light (16 h light-8 h dark) regimen.

(ii) Survival chambers. Survival studies are conducted with membrane diffusion chambers. The internal lumen of the chamber is 6 cm in diameter and 6.5 mm wide. Durapore membrane filters (0.2-p.m pore size; Millipore Corp., Bedford, Mass.) generally used instead of cellulosic membrane filters since the former are stronger and more resistant to biodegradation. A thin coating of Lubriseal is applied between the membrane filter and chamber walls to ensure a water-tight seal. The chambers and filters sterilized under UV light for at least 2 h and then assembled, with stainless-steel nuts and bolts to secure the chambers.

(iii) Experimental procedures. For all experiments, triplicate chambers are inoculated with 20 ml of washed cells suspended in water and then immersed in the above described microcosms. After certain time intervals, each chamber are removed and shaken well to suspend the cells, and 1.0 ml of suspension are removed to determine bacterial numbers. Bacteria are enumerated by the drop plate technique on LB agar plates for wild-type strains (HB101 and 50014), LB plus HgCl2 (40 pLg ml-') for E. coli 50008, or LB plus tetracycline (25 jig ml-') plus kanamycin (50 ,ug ml-') for P. putida 50058.

(iv) Sterile versus nonsterile conditions. The survivals of the genetically engineered strains under sterile and nonsterile conditions are studied by using selected chambers

inoculated with the appropriate bacterial strain and immersed in water samplers as described above.

The chambers are then replaced and thereafter represented nonsterile conditions. The water sample initially screened to ensure that none of the indigenous population grow on the selective media used for the engineered strains. This experiment is conducted at 27°C.

(v) Genetic stability. To study the stability of the engineered genes under the given test conditions, the genetically engineered strains are simultaneously enumerated on both selective and nonselective (LB) media. Stability studies are conducted at 15, 25, and 30°C under sterile conditions.

(vi) Statistical analysis. The data are expressed as mean values ± standard deviation from the mean. Basic computations are performed with the STAT-2 statistical program. Exponential decline rate models are fit to the population data by performing linear regression on logarithmic CFUs against time. Rates of population change are compared by a modified t test to determine significant differences in survival rates between bacterial strains.

Sampling procedures—(A) Ecological effects. Sampling of microcosms for routine monitoring and final sampling can be performed as follows:

(*1*) Each species of macroinvertebrates, including daphnids, ostracods, and amphipods, in the microcosm can be counted visually if the numbers of animals are less than 20 and the water is clear enough for counting. When a dense population or turbid water hampers direct counting of all macroinvertebrates in the microcosm, a series of 100–mL subsamples should be taken out of the standardized microcosm for enumeration of each macroinvertebrate species until 20 of each invertebrate are counted or 6 subsamples are removed, whichever occurs first. Water samples should be quickly captured and confined in a wide-mouth sampler before removal. Periphyton should be scraped from the glass surface and thoroughly dispersed into the culture media preceding sampling of the water column. Zooplankton should be counted in the mixed-flask microcosm by removing a series of 25 mL subsamples. Four such samples are usually sufficient. In the pond microcosm, zooplankton population should be measured twice per week. They are captured with a 2–L beaker that is submerged rapidly into the microcosm water, concentrated on a 80–m mesh plankton bucket, stained, and preserved. Population density for three groups of zooplankton, (i.e.,

cladocera, copepod, and rotifers) should be counted in the pond microcosm: major groups of zooplankton should be identified according to genus, or species if possible.

(*2*) The population density of protozoa and rotifiers should be determined in standardized aquatic microcosms, a water sample of up to 2 mL should be dispersed in a 0.01–, 0.1– or 0.2–mL aliquot on counting plates (e.g., Palmer cell with water depth of 4 mm) at 12□□magnification under a stereomicroscope. The total volume of aliquots examined should contain at least 50 individuals per species.

(*3*) The population density of each algal species can be counted twice per week. In the standardized aquatic microcosm, at least 50 cells should be counted for each known algal species from a series of up to 35 fields on the counting chamber under the microscope. If species cannot be identified, the major genus of the phytoplankton and periphyton should be identified for the following groups of algae: diatoms, green algae, euglenoid, and blue-green algae.

(*4*) Filamentous algae in the algal mat should be examined every 7 days with a microscope to detect the potential extinction of any inoculated algae and the possible presence of contaminant algal species.

(*5*) The biomass of primary producers should be estimated twice per week with in vivo fluorescence or optical density of chlorophyll *a* in acetone solution.

(*6*) The rate of uptake of dissolved inorganic carbon-14 by phytoplankton should be measured every 7 days.

(*7*) The content of chlorophyll *a* in microcosm water should be measured weekly. In this method a sample of microcosm water, from 30– to 60–mL depending on the standing crop of algae, should be sieved through a 0.3–mm nylon screen to remove any macroinvertebrates among the phytoplankton.

Distribution of the test substance among compartments of microcosms can be determined at the end of the test; the components may include:

(i) Macrophytes, subdivided into roots, shoots, and leaves.

(ii) Phytoplankton.

(iii) Zooplankton.

(iv) Benthic fauna.

(v) Sediment core, sectioned into 1–cm sub-cores.

(vi) Periphyton.

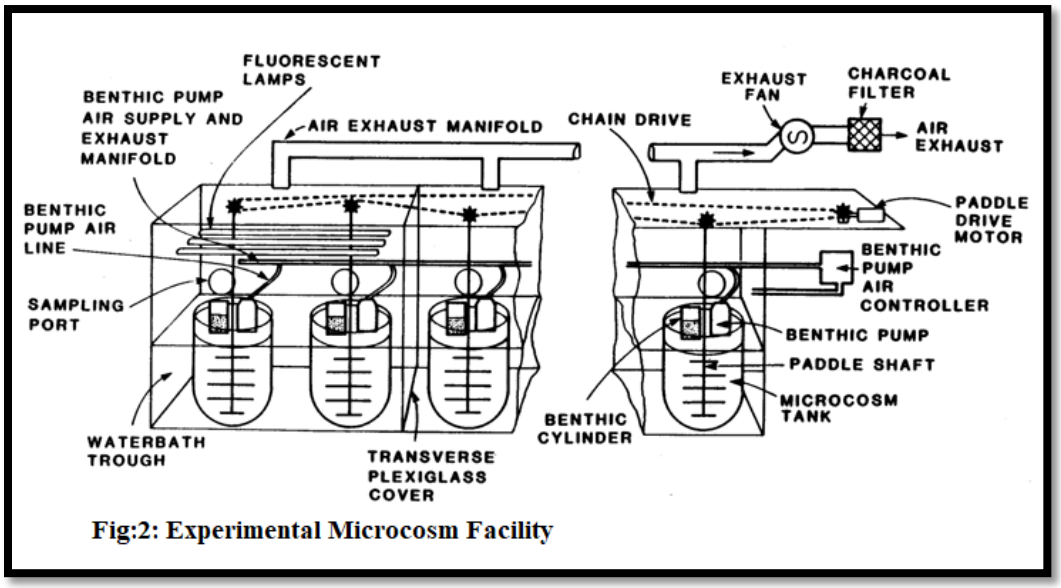


Fig:2: Experimental Microcosm Facility

Supporting equipment/facility

(A) The capacity of the water bath used to maintain the water temperature and the flow rate of the water through the water bath should be such that the water temperature in all microcosms will be kept within optimum water temperature in the natural system.

(B) Cool white fluorescent light should uniformly illuminate the water surface of all microcosms. The fluorescent lights should be mounted on a canopy above the microcosm tanks. The desired, uniform light intensity is achieved by wrapping the fluorescent lamps with aluminum foil.

(C) In the room containing the microcosms, no light source except that specifically for the microcosms should be allowed.

(D) To match the water turbulence in the natural system, the water turbulence level in the microcosms should be controlled by the speed of an electric motor that is mounted with its chain drive and drive shafts above the canopy and controls the speed of all stirring paddles.

(E) The gypsum dissolution method measures the water turbulence level by the average dissolution rate (i.e., weight loss/time) of cubes (2.5 cm 1.5 cm 1.0 cm) of pure gypsum (CaSO4) suspended in the microcosm tank or in the natural system. Gypsum cubes from the same source and lot should be used for the entire set of dissolution tests in the microcosms and in the natural system.

(F) The airspace between the canopy and water bath should be enclosed and sealed with acrylic plastic sheets to facilitate containment of the test substance transported into the gas phase (atmosphere) from the water.

(G) Airflow over the water surface (microcosms and water bath) in each compartment should be maintained by a manifold connected to an exhaust fan which draws the air from all compartments through its outlet tube and vents the exhaust air through a charcoal filter and a stack outside the laboratory building.

Microcosm. Each microcosm is a multitrophic level model that combines pelagic and benthic communities similar to those existing in the natural system.

(A) Hard glass containers are preferred to soft glass or plastic ones for the testing of organic chemicals.

(B) For each experiment, at least 20 microcosm tanks should be required. Each tank, about 140 L in capacity should hold enough water and sediment to support the quantity of benthic invertebrates present in the benthic subsystem, such as a medium-sized shellfish, for 30 days or more.

(C) The benthic cylinder, up to 30 cm tall, should have an inner diameter that makes the ratio of the sediment surface area to water volume in the microcosm equal to that in the natural system.

(D) The benthic cylinder, which holds the sediment core, should be sealed at the bottom end with a crystallization dish.

(E) The benthic pump should be an all-glass, air displacement pump. It should be large enough to provide the appropriate water flow rate over the sediment surface.

(F) To minimize disturbance of the sediment core by the discharge from the benthic pump, a diffuser should be attached to the water outlet tube of the benthic pump to direct the outgoing water into several horizontal streams over the sediment surface.

(G) If the test substance forms a thin film covering the microcosm water surface, a 6–cm length of glass cylinder, or surface film protector, should be partially submerged in the water to provide a sampling port for uncontaminated water samples after the surface film inside the cylinder is removed.

Cleaning. Microcosm tanks, benthic cylinders, crystallization dishes, benthic pumps, support rack, slick protectors, and glass rods should be cleaned before use. All equipment should be washed according to standard laboratory practices to remove any residues remaining from manufacturing or previous use. A dichromate solution should not be used for cleaning glass containers. Solvents and/or high temperature combustion may be necessary to ensure the ultimate cleanliness of the microcosms and associated glass components. If cleansing solvents are used, disposal should conform to existing standard regulations.

12.8 Batch and Continuous culture techniques

A) Batch culture technique: It is also called as **closed system of cultivation**. In this technique, first of all nutrient solution is prepared and it is inoculated with inoculums (culture organisms) and then nothing is added in the fermentation tank aeration. In this technique neither fresh medium is added nor used up media is removed from the cultivation vessels. Therefore, volume of the culture remains constant. Since the fresh media is not added during the course of incubation, concentration of nutrition decreases continuously. Furthermore various toxic metabolites also accumulate in the culture vessel. Therefore, batch culture technique gives characteristics growth curve with lag phase, log phase, stationary phase and decline phase.

Common applications for batch cultures include:

- Products that must be produced with minimal risk of contamination or organism mutation.
- Operations in which only small amounts of product are produced.
- Processes using one reactor to make various products.

- Processes in which batch or semi-continuous product separation are adequate.

Advantages of Batch culture technique: The advantages of the batch culture technique are followings:

1. Chance of contamination of the culture is minimum because it is closed system of cultivation.
2. Lower capital investment when compared to continuous processes for the same bioreactor volume.
3. More flexibility with varying product/biological systems.
4. Higher raw material conversion levels, resulting from a controlled growth period.

Disadvantages of Batch culture Technique: The disadvantages of batch culture technique are as follows:

1. Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
2. Increased focus on instrumentation due to frequent sterilization.
3. Greater expense incurred in preparing several subsultures for inoculation.
4. Higher costs for labour and/or process control for this non-stationary procedure.
5. Larger industrial hygiene risks due to potential contact with pathogenic microorganisms or toxins.

B) Continuous culture techniques:

In continuous fermentation an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system.

In a homogenously mixed bioreactor, we can have a chemostat or a turbidostat. In the chemostat, in the steady state adjusting the concentration of one substrate controls cell growth. In the turbidostat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted. In the chemostat, constant chemical environment is maintained, while in a turbidostat constant cell concentration is maintained.

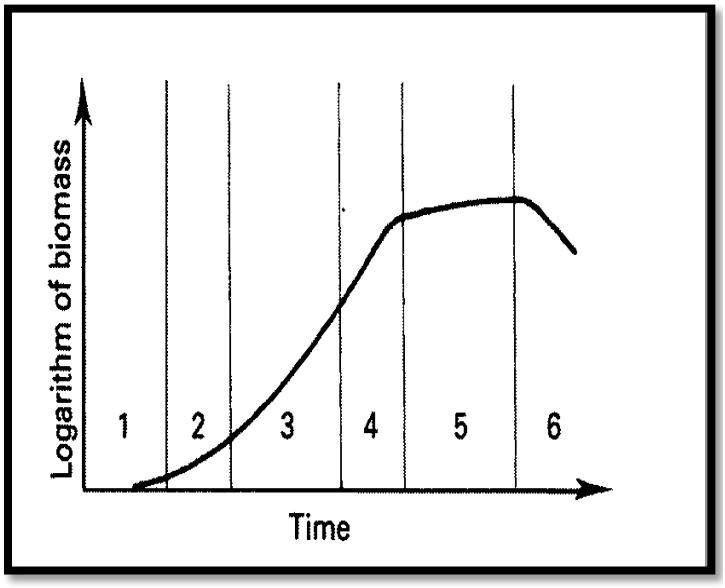


Fig-2: Growth characteristics in a batch culture of a microorganism, 1: lag phase, 2: transient acceleration, 3: exponential phase, 4: deceleration phase, 5: stationary phase, 6: death phase (Smith, 2004).

In a chemostat the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from the reservoir. The volume of fluid in the growth chamber is maintained at a constant level by some sort of overflow drain. Fresh medium is allowed to enter the growth chamber at a rate that limits the growth of the bacteria. The rate of addition of fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances and the accumulation of excess cells in the culture which are the parameters that initiate the stationary phase of the growth cycle.

Advantages of continuous culture technique:

There are several major advantages of using continuous cultures as opposed to batch cultures:

1) Continuous reactions offer increased opportunities for system investigation and analysis. As the variables remain unchanged, a benchmark can be determined for the process results, and then the effects of even minor changes to physical or chemical variables can be evaluated. By changing the growth-limiting nutrient, changes in cell composition and metabolic activity can be tracked. The constancy

of the continuous process also provides a more accurate picture of kinetic constants, maintenance energy and true growth yields.

2) Continuous culture provides a higher degree of control than a batch culture. Growth rates can be regulated and maintained for extended periods. By varying the dilution rate, biomass concentration can be controlled. Secondary metabolite production can be sustained simultaneously along with growth. In steady state continuous culture, mixed cultures can be maintained using chemostat cultures – unlike in a batch process where one organism usually outgrows another.

3) Bioreactors operated as chemostats can be used to enhance selectivity for thermophiles, osmotolerant strains or mutant organisms with high growth rates. Also the medium composition can be optimized for biomass and product formation using a pulse- and shift- method that injects nutrients directly into the chemostat. As changes are observed, the nutrient is added to the medium supply reservoir and a new steady state is established.

4) Because of the steady state of continuous culture, the results are not only more reliable but also more consistent leading to a better quality product.

5) It also results in higher productivity per unit volume, as time consuming tasks, such as cleaning and sterilization are unnecessary.

6) The ability to automate the process makes it more cost-efficient and less sensitive to the impact of human error.

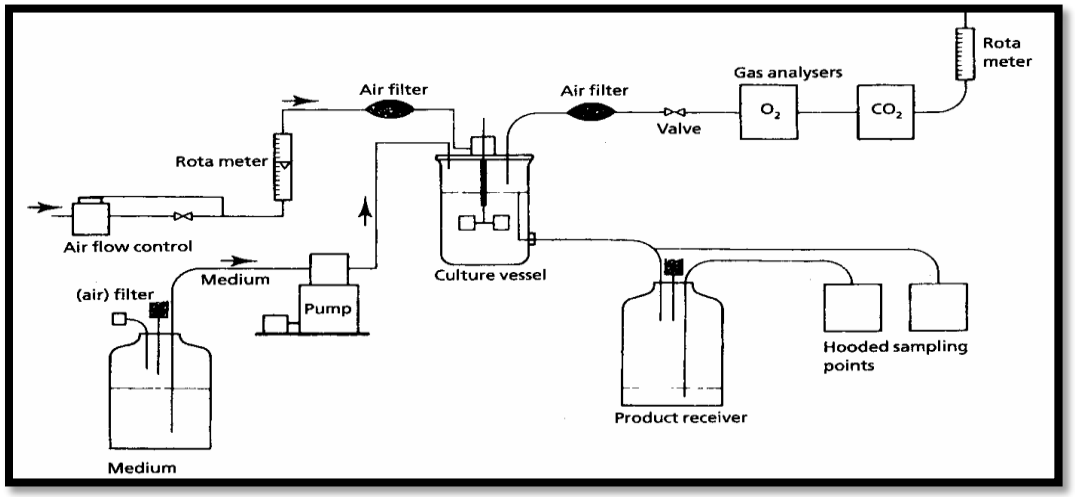


Fig-3: A simple laboratory fermenter operating on a continuous cultivation basis

(Smith, 2004).

Disadvantages of continuous culture technique: There are few disadvantages of continuous culture which are given below:

1 The control of the production of some non-growth related products is not easy. For this reason, the continuous process often requires feed-batch culturing and a continuous nutrient supply.

2 Wall growth and cell aggregation can also cause wash-out or prevent optimum steady-state growth.

3 The original product strain could be lost over time if a faster growing one overtakes it.

4 The viscosity and heterogenous nature of the mixture can also make it difficult to maintain filamentous organisms.

5 Long growth periods not only increase the risk of contamination but also dictate that the bioreactor must be extremely reliable and consistent, incurring a potentially larger initial expenditure in higher quality equipment.

Summary

In this unit we have discussed various aspects of microcosm. So far you have learnt that:

- The perfect definition of microcosm is "Microcosm means a miniaturized model of a natural ecosystem" Small-scale experimental systems also called microcosms.
- According to Carbonell and Tarazona (2014) they have historically informed understanding of ecological processes that are applicable at larger scales. Indeed, more recently, the use of microcosms has had a role in exploring the consequences of seemingly insurmountable environmental issues, such as the effect of climate change on species distributions and food-web structure, the effects of biodiversity loss on ecosystem functioning and the impacts of pollution and fisheries on ecosystems.
- Microcosms are basically small ecosystems in containers. There is a high range of microcosm studies from simple experimentally sown mixtures of two species of plants to sophisticated controlled environments housing entire terrestrial ecosystems such as the Ecotron facility at Silwood Park.
- There are various types of microcosm ASTM Method E 1197-8713.6-1, Kuehle described the NATEC test system in 1988, Litter Microcosms which was described by Van Wensem (1989), Sheppard and Evenden etc.

- There are various roles of microcosms these are used in in aquatic ecosystem analysis, in soil testing, in toxicology and pollution, in vermi-composting, in biodiversity analysis, in water pollution abatement
- There are several factors which affect the microcosm, these factors are temperature, pH, oxygen etc.
- A strain is a group of species with one or more characteristics that differentiate it from other sub group of the same species of the strain. There are various approaches for strain development such as mutant selection, recombination and recombinant DNA technology.
- Batch culture technique is also called as closed system of cultivation. In this technique, first of all nutrient solution is prepared and it is inoculated with inoculums (culture organisms) and then nothing is added in the fermentation tank aeration. Common applications for batch cultures include: products that must be produced with minimal risk of contamination or organism mutation, operations in which only small amounts of product are produced, processes using one reactor to make various products, processes in which batch or semi-continuous product separation are adequate. The advantages of the batch culture technique are chance of contamination of the culture is minimum because it is closed system of cultivation
- The disadvantages of batch culture technique are as lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor, increased focus on instrumentation due to frequent sterilization, greater expense incurred in preparing several subsultures for inoculation, higher costs for labour and/or process control for this non-stationary procedure, larger industrial hygiene risks due to potential contact with pathogenic microorganisms or toxins.
- In continuous fermentation an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system.

Terminal Questions

1 (a) Fill in the blank spaces with appropriate words.

The perfect definition of microcosm is "*Microcosm* means a ……. ……..of a natural ecosystem" Small-scale experimental systems also called **microcosms**. According to ……and ……..(2014) they have historically informed understanding of ecological processes that are applicable at larger scales. Indeed, more recently, the use of ……has had a role in exploring the consequences of seemingly insurmountable environmental issues, such as the effect of climate change on species ……..and food-web……, the effects of biodiversity loss on ecosystem functioning and the impacts of pollution and fisheries on ecosystems.

Microcosms are basically small ……in containers. There is a high range of microcosm studies from simple experimentally sown mixtures of two species of plants to sophisticated controlled environments housing entire terrestrial ecosystems such as the **Ecotron facility** at……. …….. The main role of **microcosms** is that they act as a bridge between theory and **nature**. They do not mirror nature but they can increase our understanding of natural processes by simplifying the **complexities** of our natural environment. Microcosm experiments have played an important role in developing and testing ecological theories and investigating current global change scenarios. According to **Fraser** (2004) many disciplines have utilized microcosms as a powerful research tool especially……, ecotoxicologists, ……..and soil ecologists.

2 (a) Define microcosm.

2 (b) What do you understand by microcosm? Give the different methods of microcosm

3 (a) Write a short note on role of microcosm.

3 (b) Describe the experimental design of aquatic microcosm

4 (a) What is batch culture? Explain

5 (a) Discuss about the continuous culture

6 (a) Fill the blank spaces with appropriate words

As you know ……..is a sudden and heritable change in the genes of an organism. The application of the ……..to induce a mutation is called as…….. The agent which is capable to inducing mutation is called as…….. There are various chemical mutagens such as ……agents, ……..dyes etc. When Mutation occurs without any specific treatment is called ……. ……..and mutation is resulting due to a treatment with certain agents is known as …….. ……..A change in the different species due to induced mutation is important aspect. Many mutations bring about marked changes in the biochemical characters of practical interest these are called…….. These can be used in strain development. For example *Streptomyces griseus*-Streptomycin-Mannosidostreptomycin, *Streptomyces aurofaciens* (S-604)- Produced 6-demethyl tetracycline in place of ……

6 (b) Which is closed system of culture? (Batch culture/Continuous culture/Both/None)

6 (c) Litter microcosm was described by (Van Wensem/M. Clarke/S.Lee/Darwin)

6 (d) "Microcosm means a miniaturized model of a natural ecosystem" (Yes/No)

6 (e) Describe factors affecting microcosm?

7. (a) Describe the developmental strains.

Answers

1 (a) miniaturized model, Carbonell, Tarazona, microcosms, distributions, structure, ecosystems, Silwood Park, microbiologists, limnologists

2 (a) see section 12.2.

2 (b) See section 12.2 and 12.2.1

3 (a) See section 12.2.2

3 (b) See section 12.2.5

4 (a) See section 12.2.6 in heading batch culture

5 (a) See the section 12.2.6. in heading continuous culture

6 (a) mutation, mutagens, mutagenesis, mutagens, Alkylating, Acridine, Spontaneous mutation, Induced mutation, major mutation, Tetracycline.

6 (b) Batch Culture

6 (c) Van Wensem

6 (d) Yes

6 (e) see the section 12.2.3.

7 (a) See the section 12.2.4

Unit 13 Recombinant DNA Technology

Unit Structure

13.0 Learning Objectives

After studying this unit you will be able to understand:

- What is recombinant DNA technology?
- What is procedure of recombinant DNA technology?
- What are applications of recombinant DNA technology?
- About concepts of recombinant DNA technology
- What is cloning?
- About procedure of cloning
- What are uses of cloning?
- About mutation and construction of microbial str

13.1 Introduction

As you know all the plants and animals on the earth have evolved from a common ancestor, therefore, all organisms use DNA as heredity material. Chemically DNA is similar in microscopic bacterium or a blue whale. As a result, DNA from different organisms can be "cut and pasted" together, resulting in "recombinant DNA". You have learnt about the genetic

engineering in Unit-11 of this course. The recombinant DNA technology is the basic step of genetic engineering. The first recombinant DNA molecule was produced in 1972 by Stanford.

The production of **recombinant DNA** involves cutting two different pieces of DNA with the same restriction enzyme and then joins with ligases enzymes. Today recombinant DNA technology is used extensively in research **laboratories** worldwide to explore innumerable questions about gene structure, function, **expression, regulation**, and much more. Recombinant DNA technologies are also a cornerstone of the biotechnology industry. The best example is the generation of genetically engineered plants to produce an insect toxin called ***Bt toxin***. The Bt gene is derived from a bacterium called ***Bacillus thuringiensis*** and produces a toxin that disrupts gut function in the **caterpillar larvae** of certain insects that are crop pests (which is harmful to crops). This development has had a major economic impact and reduced the expenses of pesticides used per year and has increased the longevity and success of several **crops**. Therefore, recombinant DNA technology is boon to the biological sciences. By using the recombinant DNA technology we can develop varieties of useful **plants** and **animals**. In this unit you learn about the concepts of recombinant DNA technology, cloning, mutation and construction of microbial strains.

13.2 Recombinant DNA Technology

Definitions:

- **Recombinant DNA** technology is a technology in which the molecules of DNA from two different species that are inserted into a host organism to produce new genetic combinations.
- It is deliberate, controlled manipulation of genes in an organism with the intent of making that organism better.
- It covers all various experimental techniques that manipulate the genes of the organism.
- It is joining together of DNA molecules from different organisms and inserting it into a host organism to produce new genetic combination.

- Recombinant DNA technology is series of procedures that are used to join together DNA segments.

DNA technology combines DNA from different sources to create a different sequence of DNA.

Recombinant DNA technology has great value in the field of medical, agricultural sciences and food industries. Since the focus of all genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes.

The method involves clipping the desired segment out of the surrounding DNA and copying it many times. The success of recombinant DNA technology, by which microbial cells can be engineered to produce foreign proteins, relies on the faithful reading of the corresponding genes by bacterial cell machinery, and has fueled most of the recent advances in modern molecular biology. During the last two decades, studies of cloned DNA sequences have given us a detailed knowledge of gene structure and organization, and have provided clues to the regulatory pathways by which the cell controls gene expression in the multiple cell types comprising the basic vertebrate body plan. Recent advances in this technology have also changed the course of medical research. Exciting new approaches are being developed to exploit the enormous potential of recombinant DNA research in the analysis of genetic disorders. The new ability to manipulate human genetic material has opened radically new avenues for diagnosis and treatment, and has far-reaching consequences for the future of medicine. Yet the basic principles of recombinant DNA, like the structure of DNA itself, are surprisingly simple.

13.2.1 Procedure of Recombinant DNA Technology

Steps in Recombinant DNA Technology: Basic steps of recombinant DNA technology are summarized in Fig. 1 also discussed below:

Selection and isolation of DNA insert: First step in rec DNA technology is the selection of desirable genes which is to be cloned. This desired DNA segment or gene is then isolated by enzymatic actions. The desired DNA segment is termed as **foreign DNA** or **target DNA** or **cloned DNA**. Following points must be kept in mind while selecting the foreign DNA:

It can be easily extracted from the source.

a) It can be easily introduced into the vector.

b) The desired genes should be beneficial for commercial or research point of view.

c) A number of foreign genes are being cloned for benefit of human beings. Some of these DNA inserts are the genes responsible for the production of insulin, interferon's, lymphotoxins various growth factors etc.

1. Selection of suitable cloning vector: A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be integrated. A suitable cloning vector is selected in the next step of rec DNA technology. Most commonly used vectors are plasmids. You have learnt about the plasmids in Unit- 9 of this course.

2. Introduction of DNA-insert into vector: The target DNA which has been extracted and cleaved by the restriction endonuclease enzymes are now joined by the enzyme ligase to vector DNA to form a rec DNA molecule which is often known as **cloning-vector-insert DNA construct.**

3. Recombinant DNA molecule is introduced into a suitable host: Suitable host cells are selected and the rec DNA molecule is introduced into host cells. This process of entry of rec DNA into the host cell is called **transformation**. Usually selected hosts are bacterial cells like *E. coli.*

4. Selection of transformed host cells: Transformed cells are those host cells which have taken up the rec DNA molecule. In this step the transformed cells are separated from the non-transformed cells by using various methods making use of marker genes.

5. Expression and Multiplication of DNA insert in the host: In this step, it is to be make sure that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

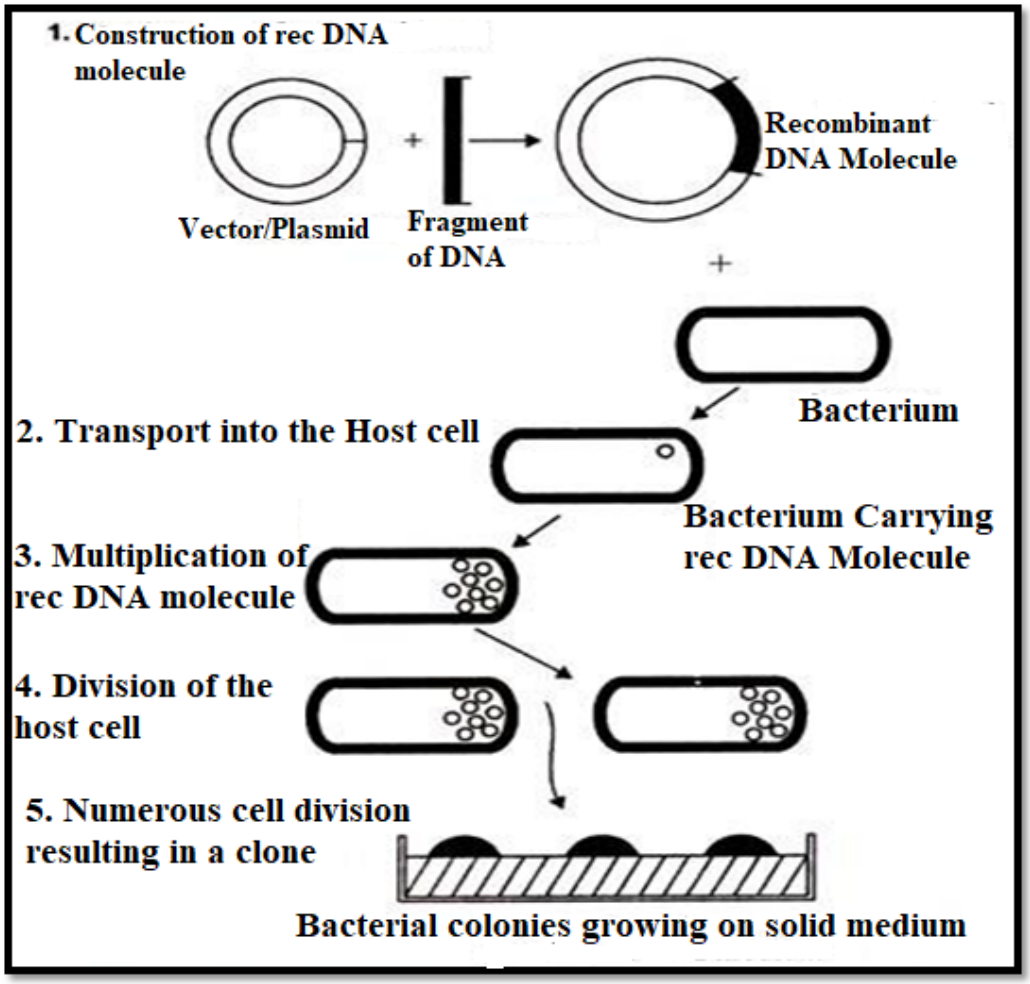


Fig-1: The basic steps of rec DNA Techno logy using the bacterial plasmid as cloning vector

Tools for Recombinant DNA Technology: There are various important biological tools which are used in rec DNA technology. These tools are enzymes, cloning vector, host plant or animal or microbe, foreign DNA and linker and adaptor sequences. These biological tools are described below:

1. Enzymes: A number of specific enzymes are utilized to achieve the objectives of rec **DNA** technology. Restriction endonucleases enzymes serve as important tools to **cut DNA** molecules at specific sites. This is the basic need for rec DNA technology. These are the enzymes that produce internal cuts (cleavage) in the strands of DNA, only within or near some specific sites called **recognition sites**/**recognition sequences**. RE enzymes are the first requirement for rec DNA technology. The presence of RE enzymes was first of all

reported by **Werner Arber** in the year **1962**. He found that when the DNA of a phage was introduced into a host bacterium, it was fragmented into small pieces. This led him to assume the presence of restriction enzymes. The first true restriction endonuclease was isolated in 1970 from the bacterium *E. coli* by **Meselson** and **Yuan**. Discovery of restriction enzyme Hind-II in 1970s by Kelly, Smith and Nathans was also important to genetic engineering. They isolated it from bacterium *Haemophilus influenza*. In the year **1978**, the Nobel Prize was awarded to **Smith, Arber** and **Nathans** for the discovery of **endonucleases**.

There are 3 types of restriction endonuclease enzymes namely Type-I Restriction Endonucleases, Type-II Restriction Endonucleases and Type-III Restriction Endonucleases. **Type I RE** are the complex type of endonucleases which cleave (break) only one strand of DNA. They require Mg++ ions and ATP for their functioning. **Type-II RE** are most important endonucleases for gene cloning and hence for rec DNA technology. These enzymes are most stable. They show cleavage only at specific sites and therefore they produce the DNA fragments of a defined length. These enzymes show cleavage in both the strands of DNA They also require Mg^{++} ions for their functioning. Such enzymes are advantageous because they don't require ATP for cleavage and they cause cleavage in both strands of DNA. Only Type II Restriction Endonucleases are used tor gene cloning due to their suitability. **Type-III RE** are not used for gene cloning. They are the intermediate enzymes between Type-I and Type-II restriction endonuclease. They require Mg^{++} ions and ATP for cleavage and they cleave the DNA at well-defined sites in the immediate vicinity of recognition sequences. **Exonuclease** is an enzyme that removes nucleotides from the ends of a nucleic acid molecule. An exonuclease removes nucleotide from the 5' or 3' end of a DNA molecule. An exonuclease never produces internal cuts in DNA.

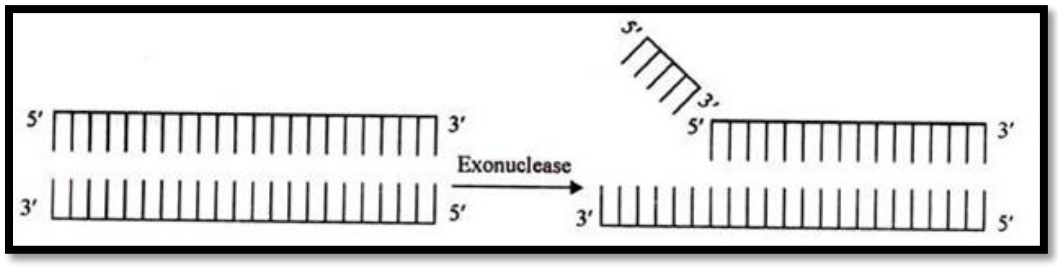


Fig-2 An Exonuclease activity: Nucleotides are removed from the end of DNA

DNA ligase enzyme joins two fragments of DNA by synthesizing the phosphodiester bond. They function to repair the single stranded nicks in DNA double helix and in rec DNA technology they are employed for sealing the nicks between adjacent nucleotides. This enzyme is also termed as **molecular glue**. **DNA polymerases** are the enzymes which synthesize a new complementary DNA strand of an existing DNA or RNA template. A few important types of DNA polymerases are used routinely in genetic engineering. **Terminal deoxynucleotidyl transferase** enzyme adds single stranded sequences to 3'-terminus of the DNA molecule. One or more deoxynbonucleotides are added onto the 3'-end of the blunt-ended fragments. **Alkaline Phosphatase** enzyme functions to remove the phosphate group from the 5'-end of a DNA molecule. **Polynucleotide Kinase** enzyme has an effect reverse to that of Alkaline Phosphatase, *i*.e. it functions to add phosphate group to the 5'-terminus of a DNA molecule. You have learnt various enzymes which are involved in genetic engineering in Unit-9 of this course.

1. Cloning Vectors: It is another important tool which is used in rec DNA technology. The cloning vector is the DNA molecule capable of replication in a host organism, into which the target DNA is introduced producing the rec DNA molecule. A cloning vector may also be termed as a **cloning vehicle**. However, under certain circumstances it becomes desirable to use different host for cloning. So, various cloning vectors have been developed based on other bacteria like *Bacillus, Pseudomonas, Agrobacterium*, etc. and on different eukaryotic organisms. The cloning vector which has only a single site for cutting by a particular restriction endormclease is considered as a good cloning vector. Different types of DNA molecules may be used as cloning vehicles such as they may be **plasmids, bacteriophages, cosmids, phasmids etc**.

2. Host Organism: A good host organism is also an essential tool tor genetic engineering. Most widely used host for rec DNA technology is the bacterium *E. coli* because cloning and isolation of DNA inserts is very easy in this host. A good host organism is the one winch easy to transform and in which the replication of rec DNA is simpler. There should not be any interfering element against the replication of rec DNA in the host cells

3. DNA Insert Or Foreign DNA: The desired DNA segment which is to be cloned is called as DNA insert or foreign DNA or target DNA. The selection of a suitable target DNA is the

very first step of rec DNA technology. The target DNA (gene) may be of viral, plant, animal or microbial origin.

4. Linker and Adaptor Sequences: Linkers and adaptors are the DNA molecules which help in the modifications of cut ends of DNA fragments. These can be joined to the cut ends and produce modifications as required. Both are short, chemically synthesized, double stranded DNA sequences. Linkers have one or more restriction endonuclease sites and adaptors have one or both sticky ends. Different types of linkers and adaptors are used for different purposes. Linkers contain target sites for the action of restriction enzymes. They can be jointed to the blunt ends of foreign DNA or vector DNA. Then they undergo a treatment with a specific restriction endonuclease to produce cohesive ends of DNA fragments EcoRI-linker is a common example of frequently used linkers.

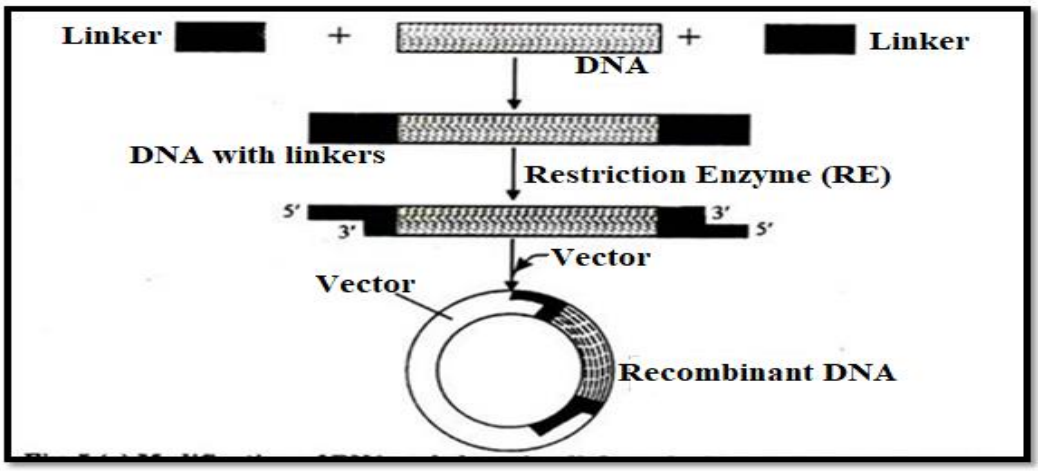


Fig:-3: Modification of DNA ends by using linkers during rec DNA technology

Adaptors are the chemically synthesized molecules which have cohesive ends. Adaptors are employed for end modification in cases where the recognition site for restriction endonuclease enzyme is present within the foreign DNA. This new molecule, so formed, is then phosphorylated at the 5 end-terminii. Finally foreign DNA modified with adaptors is integrated into the vector DNA to form the recombinant DNA molecule.

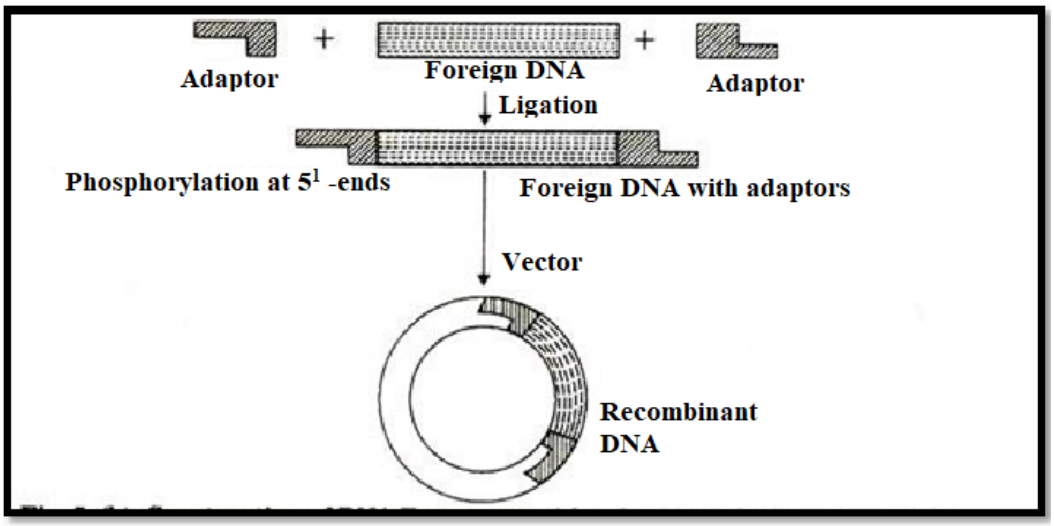


Fig-4: Construction of DNA fragments with cohesive ends using adaptors

Techniques used in Recombinant DNA Technology: A number of techniques are used for rec DNA technology. Such techniques work for the fulfillment of different requirements or to obtain proper information for drawing an exact inference during genetic engineering. Some of these important techniques are gel electrophoresis, blotting techniques, dot-blot hybridization, DNA sequencing, artificial gene synthesis, polymerase chain reaction, colony hybridization, etc. important techniques of rec DNA technology are discussed below:

1. Gel Electrophoresis: It is the technique of separation of charged molecules (in aqueous phase) under the influence of an electrical field so that they move on the gel towards the electrode of opposite charge i.e., cations move towards the negative electrode and anions move towards the positive electrode. The genomic DNA is extracted from the desired host and is then fragmented using restriction endonucleases. For separation of these cut fragments and isolation of desired DNA fragment, the technique of gel electrophoresis is employed. Gel electrophoresis may be of horizontal or vertical type. Usually agarose gel is used for separation of large segments of DNA while the polyacrylamide gel is used for the separation of small DNA fragments which are only a few base pairs long. Gel electrophoresis employs a buffer system, a medium which is a gel and a source of direct current. Samples having DNA fragments are applied on the gel and current is passed through the system for an appropriate time. Different DNA fragments move up to different distances on the gel depending on their charge to mass ratio.

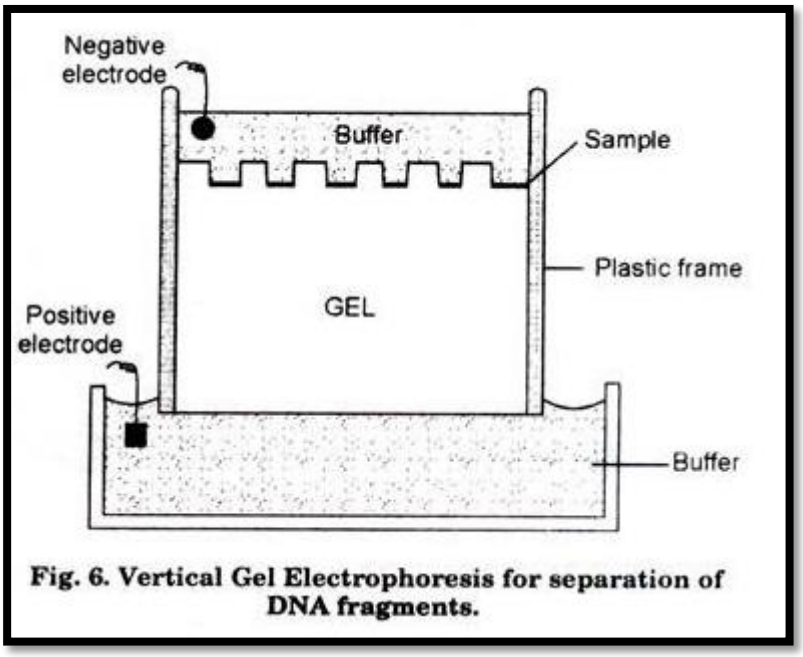


Fig. 6. Vertical Gel Electrophoresis for separation of DNA fragments.

Fig-5: Vertical Gel Electrophoresis for separation of DNA fragments

The heavier fragments move at smaller distance, while the lighter DNA fragments move to a larger distance. Following the migration of the molecules, the gel is treated with selective stains to show the location of separated molecules in the form of bands. Very large DNA molecules or chromosomes cannot be separated even by Agarose Gel electrophoresis. For separation of such very large DNA molecules, a new technique is used which is known as **Pulse Field Gel Electrophoresis** (PFGE).

2. Blotting Techniques: Visualization of a specific DNA (or RNA or protein) fragment out of many molecules requires a technique called blot transfer. In this technique, the separated bands are transferred onto a nitrocellulose membrane from the gel. Southern blotting is named after the person who devised this technique, viz. E.M. Southern (1975). The other names began as laboratory jargon but they are now accepted terms. Technically, blotting may be defined as the transfer of macromolecules from the gel onto the surface of an immobilizing membrane like nitrocellulose membrane. It is to note here that during such transfer, the relative positions of bands (of macromolecules) are same on the membrane as they occurred on the gel. The membranes which may be used in blotting are nitrocellulose membrane, nylon membrane, carboxymethyl membrane, diazobenzyl-oxymethyl (DBM) membranes, etc. Southern blotting is used for the transfer of DNA from gel onto the membrane while Northern and Western blotting are used for the transfer of RNA and protein

bands respectively. One other blotting technique is south-western blotting which examines the protein-DNA interactions. A schematic representation of southern blotting technique is given in the figure. In this technique first of all, the sample DNA is digested with restriction enzymes to obtain fragments of different lengths. These differently sized DNA segments are then passed through Agarose Gel Electrophoresis for their separation based on their lengths.

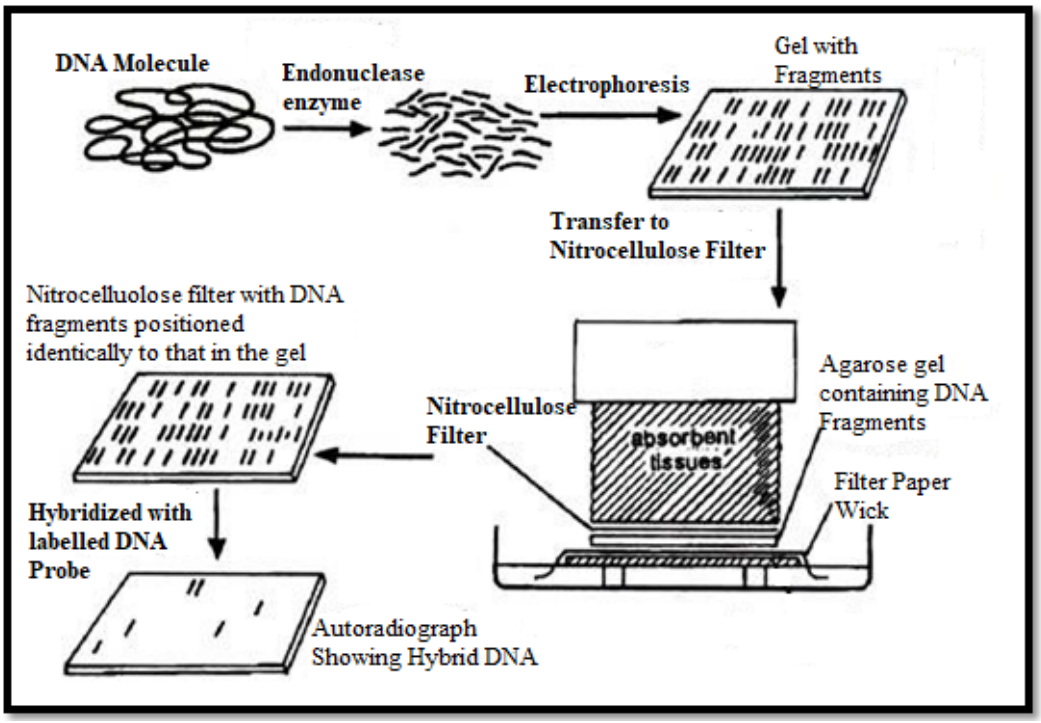


Fig-6: Method of Sothern blotting for visualizing DNA fragments

The gel so obtained with different bands of DNA fragments is placed on top of buffer saturated filter papers which act as a filter paper wick. Above gel is put a nitrocellulose filter and over nitrocellulose filter are placed many dry filter paper sheets. With the movement of buffer towards the dry filter papers, the DNA bands are also moved upwards and hence they get bound to the nitrocellulose filter membrane. Now, the nitrocellulose filter is removed and baked in vacuum. DNA fragments on the nitrocellulose filter are hybridized with single stranded radioactively labeled probes. Washing is done to remove unbound probes and finally the DNA bands with radioactivity are visualized by autoradiography. In Northern Blotting, RNA molecules are blot transferred from the gel onto a chemically reactive paper. Western blotting is used for proteins and its working is based on the specificity of antibody-

antigen reaction. In this technique the hybridization of bound proteins is done with radioactively labeled antibodies.

Colony Hybridization Technique: This technique was developed by Grunstem and Higness in the year 1975. This technique is used in genetic engineering for the identification of transformed bacterial cells (i.e. cells which contain foreign DNA). After transformation of cells with a specific DNA, it is likely that only some of those cells may have foreign DNA. For further procedure, firstly it is important to screen such cells which are having foreign DNA. This screening is done by using the technique of colony hybridization in case of bacterial cells. A similar technique namely Plaque Hybridization is utilized for screening of transformed bacteriophages. Basic principle of this technique lies in the in-situ hybridization of transformed bacterial cells with a radioactive probe sequence. Due to the specificity of probe, it enables rapid identification of one colony (through radioactivity) even amongst many thousands of colonies.

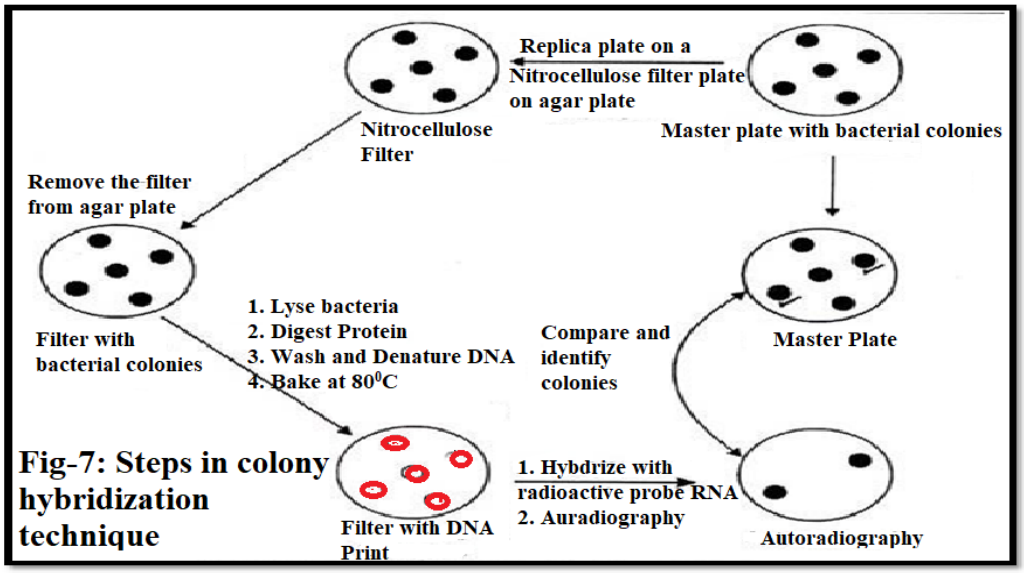


Fig-7: Steps in colony hybridization technique

The transformed bacterial cells are first of all plated on a suitable agar plate which is termed as the master plate. Colonies are grown in the master plate. These colonies on the master plate are replica-plated onto a nitrocellulose or nylon membrane by placing it gently over the master plate. This replica-plate carrying the colonies is removed and treated with alkaline reagent to lyse the bacteria. DNA of those bacterial cells is denatured. Proteins on the membrane are digested. Finally the membrane is washed to remove all other molecules,

leaving behind only the denatured DNA bound to it, in the form of DNA print of the colonies. This DNA print is then hybridized with a radioactively labeled RNA/DNA probe. Membrane is washed to remove any unbound probe and then autoradiography is done to detect radioactivity. The positions of the DNA prints showing up in autoradiograph are then compared with the master plate to identify the transformed colony. Genetic engineering or rec DNA technology has enormous and wide-spread applications in all the fields of biological sciences.

13.2.1 Uses of Recombinant DNA Technology

There are some important uses of rec DNA technology which are summarized in Fig-8 and also described below:

(1) Production of Transgenic Plants: This is one of the most important use of recombinant DNA technology. rec DNA technology produce transgenic plants or the genetically modified plants. Many transgenic plants have been developed with better traits like resistance to herbicides, insects or viruses or with expression of male sterility, etc. They allow the production of commercially important biochemical, pharmaceutical compounds, etc. Technology is capable of introducing the improved post-harvest characteristics in plants also.

(2) Production of Transgenic Animals: By the use of rec DNA technology, desired genes can be inserted into the animals to produce the transgenic animal. The method of rec DNA technology aids the animal breeders to increase the speed and range of selective breeding in case of animals. It helps for the production of better farm animals therefore, ensure more commercial benefits. Another commercially important use of transgenic animals is the production of certain proteins and pharmaceutical compounds. Transgenic animals also contribute for studying the gene functions in different animal species. Recombinant DNA technology have successfully produced transgenic animals like pigs, sheep, rats and cattle.

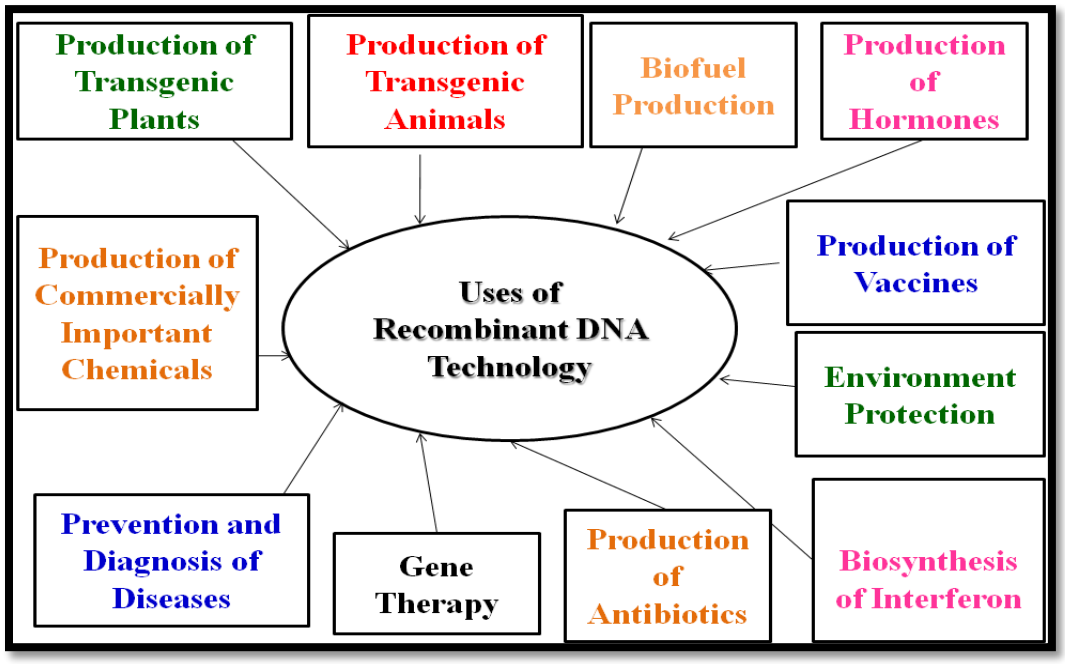


Fig-8: Showing uses of recombinant DNA Technology

(3) Production of Hormones: By the advent of techniques of rec DNA technology, bacterial cells like *E.coli* are utilized for the production of different fine chemicals like insulin, somatostatin, somatotropin and p-endorphin. Human Insulin Hormone i.e., Humulin is the first therapeutic product which was produced by the application of rec DNA technology. The desired genes are incorporated into the bacterial cells which are then cloned. Such clones are capable of producing a fair amount of hormones like insulin. These hormones have great commercial importance.

(4) Production of Vaccines: A number of vaccines have been synthesized biologically through rec DNA technology. These vaccines are effective against numerous serious diseases caused by bacteria, viruses and protozoans. These include vaccines for polio, malaria, cholera, hepatitis, rabies, smallpox, etc. The generation of DNA vaccines has revolutionized the approach of treatment of infectious diseases.

(5) Biosynthesis of Interferon: This is one of the great importances of recombinant DNA technology**.** Interferon's are the glycoproteins which are produced in virus-infected cells. Interferon's have antiviral and even anti-cancerous properties. By recDNA technology method, the gene of human fibroblasts (which produce interferon's in human beings) is inserted into the bacterial plasmid. These genetically engineered bacteria are cloned and

cultured therefore, the gene is expressed and the interferon's are produced in fairly high quantities. This interferon, so produced, is then extracted and purified.

(6) Production of Antibiotics: As you know antibiotics are very important medicines**.** Some important antibiotics are tetracyclin, penicillin, streptomycin, novobiocin, bacitracin, etc. recDNA technology helps in increasing the production of antibiotics by improving the microbial strains through modification of genetic characteristics.

(7) Production of Chemicals: Various commercially important chemicals can be produced more efficiently by utilizing the methods of rec DNA technology. A few of them are the alcohols and alcoholic beverages obtained through fermentation; organic acids like citric acid, acetic acid, etc.

(8) Application in Enzyme Engineering: As we know that the enzymes are encoded by genes, so if there are changes in a gene then definitely the enzyme structure also changes. Enzyme engineering utilizes the same fact and can be explained as the modification of an enzyme structure by inducing alterations in the genes which encode for that particular enzyme.

(9) Prevention and Diagnosis of Diseases: Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. It also provides methods for prevention of a number of diseases like AIDS, cholera, etc. Monoclonal antibodies are useful tools for disease diagnosis. Monoclonal antibodies are produced by using the technique called **hybridoma technology**. Genetic engineering allows the production of hybridoma which is a cell obtained by the fusion of a lymphocyte cell capable of producing antibodies and a single myeloma cell (tumour cell).

(10) Gene Therapy: Gene therapy is undoubtedly the most beneficial area of genetic engineering for human beings. It involves delivery of specific genes into human body to cure the diseases specially heredity diseases. Gene therapy it is the treatment of diseases by transfer and expression of a gene into the patients' cells so as to ensure the restoration of a normal cellular activity. Gene therapy is done either by using in vivo strategy (also called as patient therapy) or by using the ex vivo strategy.

(11) Uses in forensic science: The applications of rec DNA technology in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It enables us to

identify any person by analyzing his hair roots, serum, blood etc. DNA fingerprinting also helps to solve the problems of parentage and to identify the criminals.

(13) Biofuel Production: Recombinant DNA technology plays an effectively important role in a beneficial and large scale production of biofuels like biogas, bio hydrogen biodiesel bio-ethanol., etc. Genetic engineering helps to improve organisms for obtaining higher product yields and product tolerance. The fermenting microbes which are utilized for biogas production are improved at the genetic level for achieving better result.

(14) Environment Protection: Recombinant DNA technology makes its contributions to the environment protection in waste treatments and bioremediation. Major approach in environment protection is the use of recDNA technology for degradation of toxic pollutants which harm the environment. Different microbes used for sewage treatment, waste water treatment, industrial effluent treatment and for bioremediation are greatly improved by genetic engineering practices.

13.3 Cloning

Molecular cloning provides a means to exploit the rapid growth of bacterial cells for producing large amounts of identical DNA fragments, which alone have no capacity to reproduce themselves. The fragment of DNA to be amplified is first inserted into a cloning vector. The most popular vectors currently in use consist of either small circular DNA molecules (plasmids) or bacterial viruses (phage). The vectors contain genetic information that allows bacterial DNA replication machinery to copy them. **Amplification of Recombinant DNA**: The DNA segment to be amplified is separated from surrounding genomic DNA by restriction enzyme cleavage, which often produces staggered or sticky ends. In the example illustrated here, the restriction enzyme EcoRI recognizes the palindromic sequence GAATTC, and cuts on each strand between G and A (the two strands of the genomic DNA are green and purple). The plasmid vector (brown) is prepared to accept the isolated genomic DNA fragment by cutting the circular plasmid DNA at a single site with the same restriction enzyme, generating sticky ends which are complementary to the sticky ends of the genomic DNA fragment. The cut genomic DNA and the linearized plasmid are mixed together in the presence of a ligase enzyme, which rejoins the bonds in the DNA backbone on each side of the plasmid-genomic DNA junction. This recombinant DNA

molecule is then introduced into bacteria which are able to take up plasmid DNA, and then replicate the plasmid as the culture grows.

13.3.1 Procedure Of Cloning

In standard molecular cloning experiments, the cloning of any DNA fragment essentially involves seven steps: (1) Choice of host organism and cloning vector, (2) Preparation of vector DNA, (3) Preparation of DNA to be cloned, (4) Creation of recombinant DNA, (5) Introduction of recombinant DNA into host organism, (6) Selection of organisms containing recombinant DNA, (7) Screening for clones with desired DNA inserts and biological properties.

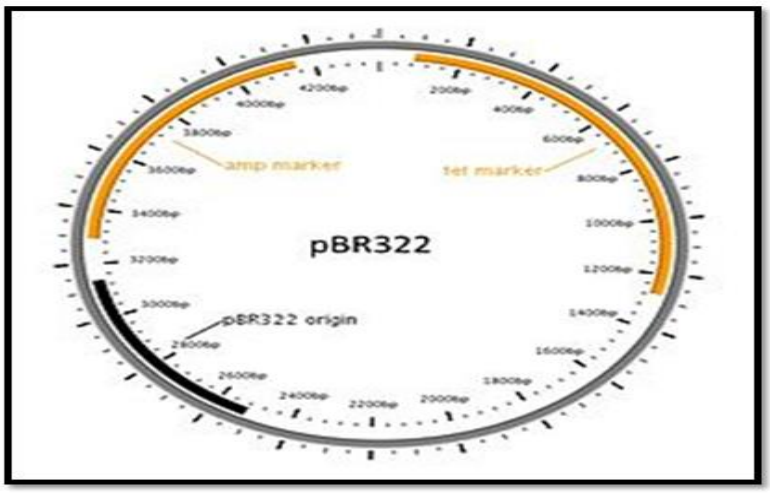


Fig-9: Diagram of a commonly used cloning plasmid; pBR322. It's a circular piece of DNA 4361 bases long. Two antibiotic resistance genes are present, conferring resistance to ampicillin and tetracycline, and an origin of replication that the host uses to replicate the DNA

Although a very large number of host organisms and molecular cloning vectors are in use, the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli* (*Escherichia coli*) and a plasmid cloning vector. *E. coli* and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available, and offer rapid growth of recombinant organisms with minimal equipment. Specialized applications may call for specialized host-vector systems. For example, if the experimentalists wish to harvest a particular protein from the recombinant organism, then an expression vector is chosen that contains appropriate signals for transcription and translation in the desired host organism. Alternatively, if replication of the DNA in different

species is desired (for example, transfer of DNA from bacteria to plants), then a multiple host range vector may be selected. In practice, however, specialized molecular cloning experiments usually begin with cloning into a bacterial plasmid, followed by subcloning into a specialized vector.

Whatever combination of host and vector are used, the vector almost always contains four DNA segments that are critically important to its function and experimental utility:

- DNA *replication origin* is necessary for the vector (and its linked recombinant sequences) to replicate inside the host organism
- one or more unique *restriction endonuclease recognition sites* to serves as sites where foreign DNA may be introduced
- a *selectable genetic marker* gene that can be used to enable the survival of cells that have taken up vector sequences
- a *tag* gene that can be used to screen for cells containing the foreign DNA

Preparation of DNA to be cloned: For cloning of genomic DNA, the DNA to be cloned is extracted from the organism of interest. Virtually any tissue source can be used as long as the DNA is not extensively degraded. The DNA is then purified using simple methods to remove contaminating proteins (extraction with phenol), RNA (ribonuclease) and smaller molecules (precipitation and/or chromatography). Polymerase chain reaction (PCR) methods are often used for amplification of specific DNA or RNA (RT-PCR) sequences prior to molecular cloning. DNA for cloning experiments may also be obtained from RNA using reverse transcriptase or in the form of synthetic DNA (artificial gene synthesis). cDNA cloning is usually used to obtain clones representative of the mRNA population of the cells of interest, while synthetic DNA is used to obtain any precise sequence defined by the designer. Such a designed sequence may be required when moving genes across genetic codes (for example, from the mitochrondria to the nucleus) or simply for increasing expression via codon optimization. The purified DNA is then treated with a restriction enzyme to generate fragments with ends capable of being linked to those of the vector. If necessary, short double-stranded segments of DNA (*linkers*) containing desired restriction sites may be added to create end structures that are compatible with the vector.

Creation of recombinant DNA with DNA ligase: The creation of recombinant DNA is in many ways the simplest step of the molecular cloning process. DNA prepared from the vector and foreign source are simply mixed together at appropriate concentrations and exposed to an enzyme (DNA ligase) that covalently links the ends together. This joining reaction is often termed ligation. The resulting DNA mixture containing randomly joined ends is then ready for introduction into the host organism. DNA ligase only recognizes and acts on the ends of linear DNA molecules, usually resulting in a complex mixture of DNA molecules with randomly joined ends. The desired products (vector DNA covalently linked to foreign DNA) will be present, but other sequences (e.g. foreign DNA linked to itself, vector DNA linked to itself and higher-order combinations of vector and foreign DNA) are also usually present. This complex mixture is sorted out in subsequent steps of the cloning process, after the DNA mixture is introduced into cells.

13.3.2 USES OF CLONING

Molecular cloning provides scientists with an essentially unlimited quantity of any individual DNA segments derived from any genome. This material can be used for a wide range of purposes, including those in both basic and applied biological science. A few of the more important applications are summarized here.

Genome organization and gene expression: Molecular cloning has led directly to the elucidation of the complete DNA sequence of the genomes of a very large number of species and to an exploration of genetic diversity within individual species, work that has been done mostly by determining the DNA sequence of large numbers of randomly cloned fragments of the genome, and assembling the overlapping sequences.

Production of recombinant proteins: Obtaining the molecular clone of a gene can lead to the development of organisms that produce the protein product of the cloned genes, termed a recombinant protein. In practice, it is frequently more difficult to develop an organism that produces an active form of the recombinant protein in desirable quantities than it is to clone the gene. This is because the molecular signals for gene expression are complex and variable, and because protein folding, stability and transport can be very challenging.

Transgenic organisms: Once characterized and manipulated to provide signals for appropriate expression, cloned genes may be inserted into organisms, generating

transgenic organisms, also termed genetically modified organisms (GMOs). Although most GMOs are generated for purposes of basic biological research (see for example, transgenic mouse), a number of GMOs have been developed for commercial use, ranging from animals and plants that produce pharmaceuticals or other compounds (pharming), herbicide-resistant crop plants, and fluorescent tropical fish for home entertainment.

Gene therapy: Gene therapy involves supplying a functional gene to cells lacking that function, with the aim of correcting a genetic disorder or acquired disease. Gene therapy can be broadly divided into two categories. The first is alteration of germ cells, that is, sperm or eggs, which results in a permanent genetic change for the whole organism and subsequent generations. This "germ line gene therapy" is considered by many to be unethical in human beings. The second type of gene therapy, "somatic cell gene therapy", is analogous to an organ transplant. In this case, one or more specific tissues are targeted by direct treatment or by removal of the tissue, addition of the therapeutic gene or genes in the laboratory, and return of the treated cells to the patient. Clinical trials of somatic cell gene therapy began in the late 1990s, mostly for the treatment of cancers and blood, liver, and lung disorders.

13.3 Mutation and Construction of Microbial Strains

Mutation: The term "Mutation" was first used by Hugo de Vries in 1901. It is its broad sense also includes the chromosomal aberration but in the most of the literatures mutation refers to only gene mutation. Gene mutation is change in the genetic material usually in DNA.

There are various types of mutation which are summarized below:

Mutation that happens naturally is called spontaneous mutation. This type of mutation is random in nature and produce genetic variation in a population. Induced mutation happens due to and man-made factor like X-ray. Mutation which occurs in somatic cells is called as Somatic mutation on the other hand if it occurs in the germinal cells, called as germinal mutation. Insertion mutation occurs when an extra nucleotide is added to DNA strand during replication. A deletion mutation occurs when a wrinkle forms on the DNA template strand and subsequently causes a nucleotide to be omitted from the replicated strand. Cystic

fibrosis is an example of this form of mutation. Some mutations affect the behavioural patterns of the organism, such as mating behavior or circadian rhythms, are called as Behaviour mutation. Mutations which interrupt the processes that are essential to survival of organism are called Lethal Mutation. Lethal mutations may categorized as recessive lethal and dominant lethal.

The agents which induced mutation in organisms, are called mutagens, these mutation may be physical or chemical. The examples of chemical mutagens include: 5-Bromouracil, Nitrous acid, Nitrosoguanidine, methyl methanesulphonate, ethyl methanesulphonate, alfatoxin B_1. Ultraviolet radiation, ionizing radiation also induced mutations in organisms.

Construction of Microbial Strains: There are various steps in construction of microbial strains which are given below:

Step-1: Isolation of Industrial Microorganisms: The first step in developing producer strains is the isolation of concerned microorganisms from their natural habitats. Alternatively, microorganisms can be obtained as pure cultures from organisations, which maintain culture collections, e.g.. American Type Culture Collection (ATCC), U.S.A. The microorganisms of industrial importance are, generally, bacteria, actinomycetes, fungi and algae. These organisms occur virtually everywhere. But most common sources of industrial microorganisms are soils, and lake and river mud. Often the ecological habitat from which a desired microorganisms is more likely to be isolated will depend on the characteristics of the product desired from it, and of process development. A variety of complex isolation procedures have been developed, but no single method can reveal all the microorganisms present in a sample. The enrichment culture techniques are designed for selective multiplication of only some of the microorganisms present in a sample. These approaches, however, take a long time (20-40 days), and require considerable labour and money. Obviously, the procedures for isolation of actinomycetes, algae, bacteria and fungi differ markedly, and they usually utilize specialized media. The main isolation methods used routinely for isolation from soil samples are as follows: sponging (soil directly), dilution gradient plate, aerosol dilution, flotation, and differential centrifugation. Often these methods are used in conjunction with an enrichment culture technique.

Step-2: Screening for New Products: The next step in developing producer strains after isolation of microorganisms is their screening. A set of highly selective procedures, which allows the detection and isolation of microorganisms producing the desired metabolite constitutes primary screening. Ideally, primary screening should be rapid, inexpensive, predictive, specific but effective for a broad range of compounds and applicable on a large scale. Primary screening is time-consuming and labour intensive since a large number of isolates have to be screened to identify a few potential ones. Rapid and accurate determination of new metabolites is necessary to avoid a wasteful duplication of effort. Computer-based databases play an important role by instantaneously providing detailed information about the already known microbial antibiotic compounds. Rapid and effective screening techniques have been devised for a variety of microbial products, which utilize either a property of the product or that of its biosynthetic pathway for detection of desirable isolates. Some of the screening techniques are relatively simple, e.g., for extracellular enzymes and enzyme inhibitors.

Table-1: A Sample of the screening approach used for detection of microbial isolates producing useful products. Of necessity, the description is generalized and highly simplified.

Product	Screen
Extracellular Enzymes	
Amylase	Starch in agar, detected by clear zones on iodine staining
Protease	Protein suspended in agar, detected by cleared zones
Lipases	Target lipid emulsified in agar, detected by cleared/zones for precipitation of liberated fatty acid by Ca^{++} in agar
Urease	pH indicator in urea agar incubator, phenol red
Enzyme inhibitors	Enzymes-containing agar, the procedure for enzyme detection is suitably modified
Antimicrobial	Primary screen is based on a broad range of specific resistant or hypersensitive microbes. Example: Penicillium and recombination deficient strains of Bacillus subtilis & E.coli for anti-tumor agents. Secondary screen uses more specialized media and conditions.
Antifungals	Chitin synthase inhibition (chitin i.e. (1→4) β-homopolymer of N-acetylglucosamine, is a major constitute of fungal cell wall)
Antiviral agents	Agar-diffusion tissue culture method for in vitro and in vivo screens using complex fermentation broths;antiviral agents pyrazomycin and mycophenolic acid identified in this manner.
Anti-tumour agents	Prescreen uses microorganisms known to be sensitive to anti-tumour agents. Example Penicillium avellaneum, mutants of Bacillus subtilis, E.coli etc. mycoplasma Acholeplasma laidlowii etc.

	Initial screens are based on in vivo animal testing which is complex and time consuming.

Suitably designed specialized screening techniques may be used to detect compounds having various pharmacological activities (other than antibiotics), some of which are listed in Table. The discovery of new microbial products may, in fact, be thought to be limited by the availability of specific, precise and rapid screening techniques for them.

Step-3: Identification of Metabolites: The initial screening is ordinarily done in plates using agar media. Subsequently, desirable isolates are screened in liquid cultures using shaker vessels since the results from plate arrays cannot always be reproduced in shake cultures. Shake cultures also allow the evaluation of different fermentation conditions, and are amenable to automated detection systems. This step allows the identification of desirable isolates and the suitable culture conditions for them. In the next step, the cultures are scaled up to 10-70 litres; these cultures provide the material necessary for developing a suitable procedure for isolation of the active principle or compound of interest by trying out various solvents-extraction procedures over a wide range of pH. The bioactivity of a metabolite is initially identified using paper chromatography or thin-layer chromatography; more recently, high performance liquid chromatography (HPLC) is more commonly and profitably used. Once the metabolite is reasonly purified, its chemical nature is determined. This information allows the researcher to decide if this metabolite in a new one.

Step-4: Maintenance of Microbial Isolates: Industrial microbiology continuously uses specific microorganism isolates/strains as research, assay, development and production cultures. These strains are highly valuable and must be preserved over long periods without any genetic and, as a result, phenotypic changes. A microbial strain discovered to produce a useful product is called research culture since it is now used for studies on product isolation and identification, strain improvement, etc. The microorganisms used for assays of the biological activities of microbial products as well as for various screening techniques are known as assay cultures. The various cultures can be maintained for reasonably prolonged periods using one of the following approaches:

1. Low temperature storage at 2-6°C on agar slants or in liquid medium; it is useful for relatively shorter periods (2-6 months) for working stock cultures.
2. Storage as frozen cultures at -20 to -100°C (both agar slants and liquid cultures).

3. Storage as lyophilized cells under vacuum at low temperatures (5°C or even -20 to -70°C). Lyophilization removes the free water from cells and spores. Protective agents like skimmed milk or sucrose reduce the detrimental effects of lyophilization. Lyophilization is easily done by using automated freeze-drying machines in which the cells are first frozen and then their free water is withdrawn under high vacuum.
4. Storage of vegetative cells/spores in liquid nitrogen (-196°C) or in the vapour phase of liquid nitrogen (-167°C).
5. Air dried at room temperature on sterile loam sand or on other natural substrates like maize seed, rice bran, etc., and stored at room temperature or in a refrigerator. Bacterial cultures may remain viable up to 70-80 years. It is more suited for organisms that form spores.
6. Storage in glycerine stabs (0.85 ml of cell suspension mixed with 0.15 ml of sterile glycerol and stored at -70 or -75°C). This is commonly used for recombinant bacteria.

In contrast, primary stock cultures are kept in long-term storage for later use; they are used to produce new working stock cultures, as per need. A stock culture may be simply defined as a culture, which serves as a source of inoculum.

Step-5: Strain Improvement: After an organism producing a valuable product is identified, it becomes necessary to increase the product yield from fermentation to minimize production costs.

Product yields can be increased by the following strategies:

- Developing a suitable medium for fermentation,
- Refining the fermentation process, and
- Improving the productivity of the strain.

Generally, major improvements arise from the last approach; therefore, all fermentation enterprises place a considerable emphasis on this activity. The techniques and approaches used to genetically modify strains to increase the production of the desired product is called strain improvement or strain development.

Strain improvement is based on the three-approach namely Mutant selection and selective isolation of mutants (secondary screening), recombination and Recombinant DNA technology.

A. Mutant Selection and Selective Isolation of Mutants (Secondary Screening): Large scale mutant selection programmes begin when favourable reports of clinical trials are obtained. In the early stages, selection of spontaneous mutants may be helpful, but induced mutations are the most common sources of improvements. Mutations occurring without any specific treatment are called spontaneous mutation, while those resulting due to a treatment with certain agents are known as induced mutations, the agents being referred to as mutagens. Application of mutagens to induce mutations is called mutagenesis. Either physical or chemical mutagens can be employed. Usually, the frequency of mutants with desirable phenotype is quite low; hence the major bottleneck is the identification and isolation of such cells from among the large number of non- mutant/undesirable mutant cells. A mutation is a sudden and heritable change in the traits of an organism. Many mutations bring about marked changes in the biochemical characters of practical interest; these are called major mutations. Some of the major mutations can be useful in strain improvement. For example, the original strain of *Streptomyces griseus* produced small amounts of streptomycin and large amounts of mannosido-streptomycin, which has low antibiotic activity.

A major mutant isolated from this strain produced negligible amounts of mannosidostreptomycin and much larger quantities of streptomycin. Similarly, a mutant strain (S-604) of *Streptomyces aureofaciens* produces 6-demethyl tetracycline in place of tetracycline; this demethylated form of tetracycline is the major commercial form of tetracycline. In contrast, most improvements in biochemical production have been due to the stepwise accumulation of so called minor genes. These genes lead to small increases (or decreases) in the antibiotic or other biochemical production, and selection may be expected to result in a 10-15% increase in yield.

The selected strains are usually subjected to successive cycles of mutagenesis and selection, and after several cycles large increases in yields are likely to be obtained. In some cases, improvements have been obtained even without the use of mutagens.

A majority of desirable mutants, especially the 'minor gene' mutants, showing increased production, are isolated by screening a large number of clones surviving the mutagen treatment; this is called selective isolation of mutants (secondary screening). But this approach requires a large amount of work.

Therefore, efforts have increasingly focussed on developing techniques for the isolation of particular classes of mutants, which are likely to be overproducers.

Some of the relevant strategies are briefly summarized below; the selection for these classes of mutants is simple, easy and effective:

- Isolation of auxotrophic mutants is the basis for commercial amino acid production in Japan from the bacterium Corynebacterium glutamicus. An auxotrophic mutant has a defect in one of its biosynthetic pathways so that it requires a specific biochemical for normal growth and development.
- Many analogue-resistant mutants have feed-back insensitive enzymes of the biosynthetic pathway the analogue of whose product was used for selection of such cells. In feed-back inhibition, activity of an enzyme is inhibited by the end-product of the biosynthetic pathway in which the enzyme participates.
- Sometimes revert-ants from nonproducing mutants of a strain are high producers, e.g., one such reversion mutant of Streptomyces viridifaciens showed over 6-fold increase in chlortetracycline production over the original strain from which the nonproducing mutant was obtained. When a mutant mutates back to its original phenotype it is called reversion, and the mutant is known as revertant, e.g., non-producer mutant mutating back to producer phenotype.
- Reversion mutants of appropriate auxotrophs may often be high producers, e.g., in case of S. viridifaciens reversion mutants of an auxotrophic mutant requiring homocysteine showed 28% more chlortetracycline yield than the original strain.
- In some cases, selection for resistance to the antibiotic produced by the organism itself may lead to increased yields. For example, Streptomyces aureofaciens mutants selected for resistance to 200-400 mg/l chlortetracycline showed a four-fold increase in the production of this antibiotic.

- Sometimes, mutants with altered cell membrane permeability show high production of some metabolites. A mutant E. coli strain has defective lysine transport; it actively excretes L-lysine into the medium to 5-times as high concentration as that within its cells.
- Mutants have been selected to produce altered metabolites, especially in case of aminogycoside antibiotics. For example. Pseudomonas aureofaciens produces the antibiotic pyrrolnitrin; a mutant of this organism yields 4'-fluoropyrrolnitrin.

B. Recombination: Recombination may be defined as formation of new gene combinations among those present in different strains. This approach has been highly successful in the improvement of animals and plants. Once several different mutants have been isolated, recombination is used for both genetic analysis as well as strain improvement. It is used to bring the desirable alleles present in two or more strains into a single strain to increase product yields or to generate new products. Recombination may be based on Sexual reproduction, Parasexual cycle or Protoplast fusion.

Table-2: A summary of some of the types of changes in metabolite production induced by recombinant DNA Technology or genetic engineering

Approach	Features	Example/Remarks
Recombinant DNA Technology	Genes from other organisms transferred into microorganism	New genes transferred; entirely new products, modified products, enhanced products yields etc.
Recombinant proteins	Proteins encoded by the transgenes are the products of interest	Insulin, interferon etc.
Metabolic engineering	Metabolites generated by the transgene encoded enzymes are the products of interest	Existing metabolic pathways modified, extended , made more efficient or new pathways introduced
i. Product modification	The new enzymes modifies the product of existing biosynthetic pathway	Conversion of cephalosporin C into 7-aminocephalosporanic acid by D-amino acid oxidase
ii. New substrate utilization	Inaccessible substrate converted into accessible form.	Beer fermentation by yeast; cyclodextrins converted into glucose which is utilized by yeast.

iii. Completely new metabolite	All the genes of a new pathway transferred	E. coli transfer of two genes for polyhydroxybutyrate synthesis from alcaligenes eutrophus
iv. Enhanced metabolite production	Amplification of the gene encoding that enzyme whose activity is rate limiting	Gene cefEF of C. acremonium catalyzing the conversion of penicillin N; increased cyclosporin yield.
v. Enhanced growth	Enhanced substrate utilization	*E.coli* glutamate dehydrogenase into M. methylotrophs carbon conversion increased from 4% to 7%

Summary

In this unit we have discussed various aspects of recombinant DNA Technology: Concepts, cloning mutation and construction of microbial strains. So far you have learnt that:

- Recombinant DNA technology is a technology in which the molecules of DNA from two different species that are inserted into a host organism to produce new genetic combinations.
- Recombinant DNA technology is series of procedures that are used to join together DNA segments.
- DNA technology combines DNA from different sources to create a different sequence of DNA.
- Basic steps of recombinant DNA technology are Selection and isolation of DNA insert, Selection of suitable cloning vector, Introduction of DNA-insert into vector, Recombinant DNA molecule is introduced into a suitable host, Selection of transformed host cells and Expression and Multiplication of DNA insert in the host.
- There are various important biological tools which are used in rec DNA technology. These tools are enzymes, cloning vector, host plant or animal or microbe, foreign DNA and linker and adaptor sequences.
- A number of techniques are used for rec DNA technology. Such techniques work for the fulfillment of different requirements or to obtain proper information for drawing an exact inference during genetic engineering. Some of these important techniques

are gel electrophoresis, blotting techniques, dot-blot hybridization, DNA sequencing, artificial gene synthesis, polymerase chain reaction, colony hybridization, etc.

- There are some important uses of rec DNA technology which are : Production of Transgenic Plants, Production of Transgenic Animals, Production of Hormones, Production of Vaccines, Biosynthesis of Interferon, Production of Antibiotics, Production of Chemicals, Application in Enzyme Engineering, Prevention and Diagnosis of Diseases, Gene Therapy etc.
- Molecular cloning provides a means to exploit the rapid growth of bacterial cells for producing large amounts of identical DNA fragments, which alone have no capacity to reproduce themselves. The fragment of DNA to be amplified is first inserted into a cloning vector. The most popular vectors currently in use consist of either small circular DNA molecules (plasmids) or bacterial viruses (phage). The vectors contain genetic information that allows bacterial DNA replication machinery to copy them.
- The term “Mutation” was first used by Hugo de Vries in 1901. It is its broad sense also includes the chromosomal aberration but in the most of the literatures mutation refers to only gene mutation. Gene mutation is change in the genetic material usually in DNA.
- There are various types of mutation which are as Mutation that happens naturally is called spontaneous mutation. This type of mutation is random in nature and produce genetic variation in a population. Induced mutation happens due to and man-made factor like X-ray. Mutation which occurs in somatic cells is called as Somatic mutation on the other hand if it occurs in the germinal cells, called as germinal mutation.
- Insertion mutation occurs when an extra nucleotide is added to DNA strand during replication. A deletion mutation occurs when a wrinkle forms on the DNA template strand and subsequently causes a nucleotide to be omitted from the replicated strand. Cystic fibrosis is an example of this form of mutation. Some mutations affect the behavioural patterns of the organism, such as mating behavior or circadian rhythms, are called as Behaviour mutation.

- Mutations which interrupt the processes that are essential to survival of organism are called Lethal Mutation. Lethal mutations may categorized as recessive lethal and dominant lethal.
- The agents which induced mutation in organisms, are called mutagens, These mutation may be physical or chemical. The examples of chemical mutagens include : 5-Bromouracil, Nitrous acid, Nitrosoguanidine, methyl methanesulphonate, ethyl methanesulphonate, alfatoxin B1. Ultraviolet radiation, ionizing radiation also induced mutations in organisms.
- There are various steps in construction of microbial strains which are Isolation of Industrial Microorganisms, Screening for New Products, Identification of Metabolites, Maintenance of Microbial Isolates, Strain Improvement.
- Strain improvement is based on the three-approach namely Mutant selection and selective isolation of mutants (secondary screening), recombination and Recombinant DNA technology.

Terminal Questions

1 (a) Fill in the blank spaces with appropriate words.

The production of **recombinant DNA** involves cutting two different pieces of DNA with the same restriction enzyme and then joins with ligases enzymes. Today recombinant DNA technology is used extensively in research **laboratories** worldwide to explore innumerable questions about gene structure, function, **expression, regulation**, and much more. Recombinant DNA technologies are also a cornerstone of the biotechnology industry. The best example is the generation of genetically engineered plants to produce an insect toxin called ***Bt toxin***. The Bt gene is derived from a bacterium called ***Bacillus thuringiensis*** and produces a toxin that disrupts gut function in the **caterpillar larvae** of certain insects that are crop pests (which is harmful to crops). This development has had a major economic impact and reduced the expenses of pesticides used per year and has increased the longevity and success of several **crops**. Therefore, recombinant DNA technology is boon to the biological sciences. By using the recombinant DNA technology we can develop varieties of useful **plants** and **animals**.

2 (a) Give the definitions of rec DNA technology.

2 (b) What do you understand by recombinant DNA technology? Give the procedure of recombinant DNA technology.

3 (a) Describe tools for recombinant DNA technology.

3 (b) Write about gel electrophoresis.

4 (a) What is blotting technique? Explain

5 (a) Discuss about the colony hybridization

6 (a) Fill the blank spaces with appropriate words.

A number of specific enzymes are utilized to achieve the objectives of rec **DNA** technology. Restriction endonucleases enzymes serve as important tools to **cut DNA** molecules at specific sites. This is the basic need for rec DNA technology. These are the enzymes that produce internal cuts (cleavage) in the strands of DNA, only within or near some specific sites called **recognition sites**/**recognition sequences**. RE enzymes are the first requirement for rec DNA technology. The presence of RE enzymes was first of all reported by **Werner Arber** in the year **1962**. He found that when the DNA of a phage was introduced into a host bacterium, it was fragmented into small pieces. This led him to assume the presence of restriction enzymes. The first true restriction endonuclease was isolated in 1970 from the bacterium *E. coli* by **Meselson** and **Yuan**. Discovery of restriction enzyme Hind-II in 1970s by Kelly, Smith and Nathans was also important to genetic engineering. They isolated it from bacterium *Haemophilus influenza*. In the year **1978**, the Nobel Prize was awarded to **Smith, Arber** and **Nathans** for the discovery of **endonucleases**.

6 (b) Foreign DNA is also known as (mDNA/cloned DNA/T-DNA/B-DNA)

6 (c) First rec DNA molecule was produced by (Stanford/D. Lehman/Morgan/Griffith)

6 (d) Which one of the following is/are cloning vector/s? (Plasmids/Bacteriophages/Cosmid/All of the above)

6 (e) What are the uses of rec DNA technology? Explain in detail

7 (a) Describe the procedure of cloning.

7 (b) What is mutation? Explain

7 (c) Describe various steps which are involved in construction of microbial strains.

Answers

1 (a); 2 (a) see the section 13.2; (b) See the section 13.2.1; 3 (a) See the section 13.2.1 under heading tools for rec DNA technology; 3 (b) See the section 13.2.1 under the heading gel electrophoresis; 4 (a) See the section 13.2.1 under the heading blotting technique; 5 (a) See the section 13.2.1 under the heading colony hybridization; 6 (a); 6 (b) Cloned DNA; 6 (c) Stanford; 6 (d) All of the above; 6 (e) See the section 13.2.2; 7 (a) See the section 13.3, 13.3.1 and 13.3.2; 7 (b) See the section 13.4. under heading mutation; 7 (c) See the section 13.4 under the heading construction of microbial strains.