**B.Sc.Microbiology** **2018-2019**

**18MBU403** **ENVIRONMENTAL MICROBIOLOGY** **Semester – IV (4H – 4C)**

**Instruction Hours / week:L: 4 T: 0 P: 0** **Marks:** Internal: **40** External: **60** Total: **100**
**End Semester Exam:** 3 Hours

## COURSE OBJECTIVES

- To educate students about Environmental monitoring and environmental aspects of microbes.
- To impart a knowledge on Microbes and environment and ecological importance.

## COURSE OUTCOME

It provides a comprehensive overview of biogeochemical processes relevant to environmental scientists and engineers mediated by microorganisms.

**Unit I**

Structure and function of ecosystems (Definition and concept). Types of Environment -Terrestrial, Aquatic and extreme habitats. Environmental factors affecting microbial growth. Microbial succession in decomposition of plant organic matter.

**Unit II**

Microbe interactions: Mutualism, synergism, commensalism, competition, amensalism, parasitism, predation. Microbe-Plant interaction: Symbiotic and non-symbiotic interactions. Microbe-animal interaction: Microbes in ruminants, nematophagus fungi and symbiotic luminescent bacteria

**Unit III**

Carbon cycle: Microbial degradation of cellulose, hemicelluloses, lignin and chitin. Nitrogen cycle: Nitrogen fixation, ammonification, nitrification, denitrification and nitrate reduction. Phosphorus cycle: Phosphate immobilization and solubilisation. Sulphur cycle: Microbes involved in sulphur cycle. Other elemental cycles: Iron and manganese

**Unit IV**

Solid Waste management: Sources and types of solid waste, Methods of solid waste disposal (composting and sanitary landfill and incineration). Liquid waste management: Composition and strength of sewage (BOD and COD), Primary, secondary (oxidation ponds, trickling filter, activated sludge process and septic tank) and tertiary sewage treatment.

**Unit V**

Principles and biodegradation of common pesticides, organic (hydrocarbons, oil spills) and inorganic (heavy metals-chelation) matter, biosurfactants. Treatment and safety of drinking (potable) water, methods to detect potability of water samples: (a) MPN test (b) Membrane filter technique. GMO and their impact.

## SUGGESTED READINGS


1. Maier RM, Pepper IL and Gerba CP. (2009). Environmental Microbiology. 2$^{nd}$ edition, Academic Press
2. Okafor, N (2011). Environmental Microbiology of Aquatic & Waste systems. 1$^{st}$ edition, Springer, New York
3. Atlas RM and Bartha R. (2000). Microbial Ecology: Fundamentals & Applications. 4$^{th}$ edition. Benjamin/Cummings Science Publishing, USA
4. Subba Rao NS. (1999). Soil Microbiology. 4$^{th}$ edition. Oxford & IBH Publishing Co. New Delhi.

5. Barton LL & Northup DE (2011). Microbial Ecology. 1st edition, Wiley Blackwell, USA Campbell RE. (1983). Microbial Ecology. Blackwell Scientific Publication, Oxford, England.
6. Coyne MS. (2001). Soil Microbiology: An Exploratory Approach. Delmar Thomson Learning.
7. Lynch JM & Hobbie JE. (1988). Microorganisms in Action: Concepts & Application in Microbial Ecology. Blackwell Scientific Publication, U.K.
8. Martin A. (1977). An Introduction to Soil Microbiology. 2nd edition. John Wiley & Sons Inc. New York & London.
9. Stolp H. (1988). Microbial Ecology: Organisms Habitats Activities. Cambridge University Press, Cambridge, England.
10. Madigan MT, Martinko JM and Parker J. (2014). Brock Biology of Microorganisms. 14th edition. Pearson/ Benjamin Cummings.

| LECTURE PLAN-UNIT-1 | | | |
|---|---|---|---|
| S.NO | Lecture duration hour | Topics | Supporting materials |
| 1 | 1 | Introduction to environmental microbiology | T2 121-122,T11-4 |
| 2 | 1 | Structure and function of ecosystem | R2 18-37 |
| 3 | 1 | Types of environment-terrestrial soil flora | R2 647-650 |
| 4 | 1 | Aquatic environment micro flora in fresh water | R2 644-650 |
| 5 | 1 | Aquatic environment micro flora in marine habitat | T1 343-350 |
| 6 | 1 | Microbes in human and animal body | R2 583-585, R2 699-704 |
| 7 | 1 | Environmental factors affecting microbial growth | R2 624 |
| 8 | 1 | Microbial succession in decomposition of plant organic matter | T1 188-189 |
| 9 | 1 | Unit Revision | |
| Textbooks: | | T1- Microbial Ecology: Fundamentals & Applications – Atlas Bartha (2000) 4th Edition,T2-Microbial ecology- Nicolas.S.Panikov(2010). | |
| Journals: | | - | |
| Website: | | - | |
| Reference books: | | R1-Fudamentals of ecology– Odum Ep & Barret GW. 5th Edition<br>R2- Microbiology. Prescot, Harley & Klein.6th Edition | |

| LECTURE PLAN-UNIT-2 | | | |
|---|---|---|---|
| S.NO | Lecture duration hour | Topics | Supporting materials |
| 1 | 1 | Microbial Interactions.mutualism,synergism,commensalism | T1 70-101 |
| 2 | 1 | Microbial interactions. competition,amensalism,parasitism and predation | T1 70-101 |
| 3 | 1 | Microbe plant interaction-symbiotic interaction | T1 119-123 |
| 4 | 1 | Microbe plant interaction-non symbiotic interaction | T2 109-118 |
| 5 | 1 | Microbe animal interaction | R2 583-585 |
| 6 | 1 | Microbes in ruminants | R2 583-585 |
| 7 | 1 | Microbes in nematophagus fungi | T1 167-169 |
| 8 | 1 | Microbes in symbiotic luminiscent bacteria | T2 170-171 |
| 9 | 1 | Unit Revision | |
| Textbooks: | | T1- Microbial Ecology: Fundamentals & Applications – Atlas Bartha (2000) 4th Edition | |
| Journals: | | - | |
| Website: | | - | |
| Reference books: | | R1-Fudamentals of ecology– Odum Ep & Barret GW. 5th Edition<br>R2- Microbiology. Prescot, Harley & Klein.6th Edition | |

| LECTURE PLAN-UNIT-3 | | | |
|---|---|---|---|
| S.NO | Lecture duration hour | Topics | Supporting materials |
| 1 | 1 | Introduction-Biogeochemical cycle | R1-1222-1223 |
| 2 | 1 | carbon cycle.Microbial degradation of cellulose,Hemicellulose | R1-1222-1223 |
| 3 | 1 | Carbon cycle.Microbial degradation of lignin and chitin | T1-413-415 |
| 4 | 1 | Nitrogen cycle. | T1- 427-429 |
| 5 | 1 | Nitrogen Fixation,ammonification | T1- 430-431 |
| 6 | 1 | Denitrification and nitrate reduction | R2-431-450 |
| 7 | 1 | Phosphorous Cycle:Phosphate immobilisation and solubilisation. | T1- 447-450 |
| 8 | 1 | Microbes involved in sulphur cycle | T1 435-446 |
| 9 | 1 | Iron and manganese cycle. | T1-450-456 |
| 10 | 1 | Unit Revision | |
| Textbooks: | | T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4th Edition. | |
| Journals: | | - | |
| Website: | | - | |
| Reference books: | | T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4th Edition. | |

| LECTURE PLAN-UNIT-4 | | | |
|---|---|---|---|
| S.NO | Lecture duration hour | Topics | Supporting materials |
| 1 | 1 | Introduction solid waste Management | R1 600-601 |
| 2 | 1 | Solid waste management. Sources and types of solid waste | R1 602 |
| 3 | 1 | Methods of solid waste disposal-Composting | T1-413-414 |
| 4 | 1 | Methods of solid waste disposal-landfill and incineration | T1-414-415 |
| 5 | 1 | Liquid waste management Composition and strength of sewage BOD | W1- 2-4 |
| 6 | 1 | Liquid waste management Composition and strength of sewage COD | W1-5 |
| 7 | 1 | Primary and Secondary treatment oxidation ponds,trickling filter. | R2 602-610 |
| 8 | 1 | Activated sludge process and septic tank | R2-608-610 |
| 9 | 1 | Tertiary sewage treatment | R2- 611-614 |
| 10 | 1 | Unit Revision | |
| Textbooks: | | T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4th Edition. | |
| Journals: | | - | |
| Website: | | Nptel.ac.in/courses | |
| Reference books: | | R1-Fudamentals of ecology– Odum Ep & Barret GW. 5th Edition<br>R2- Microbiology- Pelzar, Chan, Kries 5th Edition | |

| LECTURE PLAN-UNIT-5 | | | |
|---|---|---|---|
| S.NO | Lecture duration hour | Topics | Supporting materials |
| 1 | 1 | Introduction-biodegradation | R1 |
| 2 | 1 | Principles and Biodegradation of common pesticides | R1 |
| 3 | 1 | Organic(hydrocarbons,oil spills) | T1-413-415 |
| 4 | 1 | Inorganic(Heavy metals chelation)matter,biosurfactants. | W1- 2-4 |
| 5 | 1 | Treatment and safety drinking water | W1-5 |
| 6 | 1 | Methods to detect potability of water samples.MPN test | R2 602 |
| 7 | 1 | Methods to detect potability of water samples membrane filter technique | R2 602-610 |
| 8 | 1 | GMO and their impact | R2-608-610 |
| 9 | 1 | Unit Revision | |
| 10 | 1 | Unit Revision | |
| Textbooks: | | T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4th Edition | |
| Journals: | | - | |
| Website: | | Nptel.ac.in/courses | |
| Reference books: | | R1-Fudamentals of ecology– Odum Ep & Barret GW. 5th Edition<br>R2- Microbiology- Pelzar, Chan, Kries 5th Edition | |

## INTRODUCTION

The word *ecology* was coined by the German zoologist Ernst Haeckel, who applied the term *oekologie* to the "relation of the animal both to its organic as well as its inorganic environment." The word comes from the Greek *oikos*, meaning "household, home, or place to live." Thus, ecology deals with the organism and its environment. The word *environment*
includes both other organisms and physical surroundings. It involves relationships between individuals within a population and between individuals of different populations.*Microbial ecology* is the science that specifically examines the relationship between microorganisms and their biotic and abiotic environment. Like plant, animal, and human
ecology, microbial ecology applies the general ecological principles to explain life functions of microorganisms in situ, i.e., directly in their natural environment rather than simulated under artificial laboratory conditions ex situ or in vitro. Although the in situ microbial processes are the ultimate goal in the majority of ecological studies, it does not exclude
laboratory experiments and mathematical modeling as efficient research tools at intermediate stages aimed at the elucidation of underlying mechanisms and testing hypothesis.

**Ecosystems: Concept, Structure and Functions of Ecosystems. Concept of an Ecosystem:**

Living organisms cannot live isolated from their non•living environment because it provides materials and energy for the survival of living organisms i.e. there is interaction between a biotic community and its environment to produce a stable system; a natural self•sufficient unit which is known as an ecosystem.

**What is the structure of the ecosystem?**

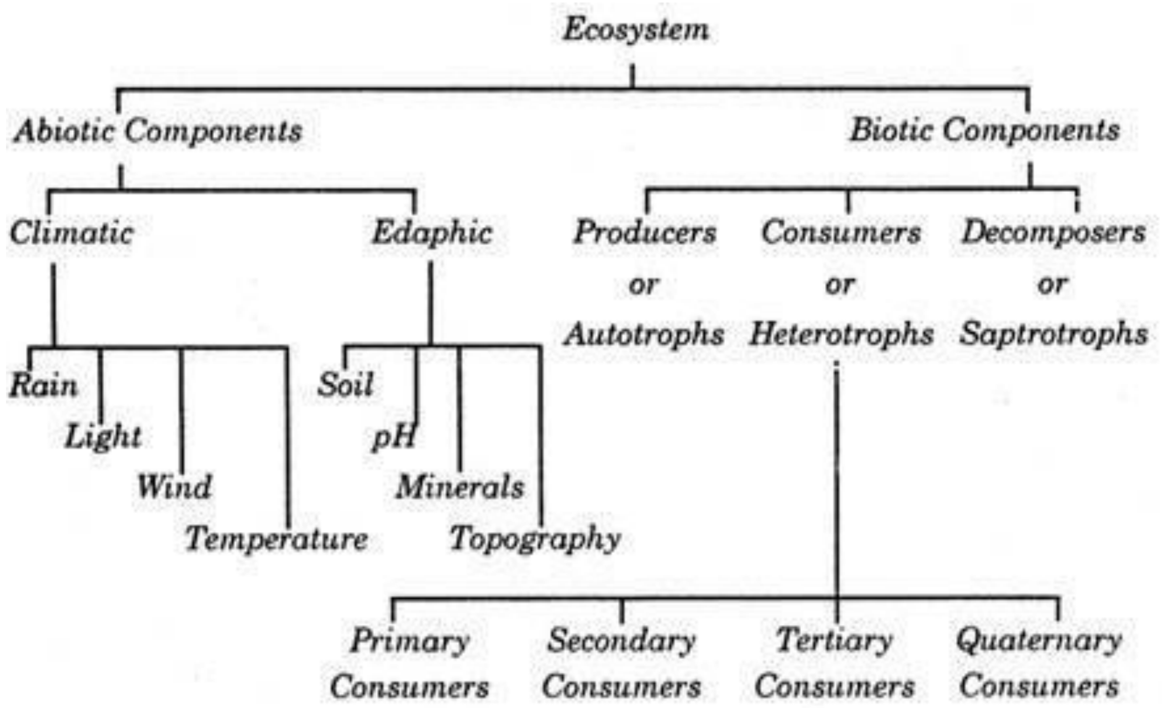
An ecosystem is a community of living **organisms** in conjunction with the **nonliving** components of their environment (things like air, water and mineral soil), interacting as a system. These biotic and abiotic components are regarded as linked together through nutrient cycles and energy flows.

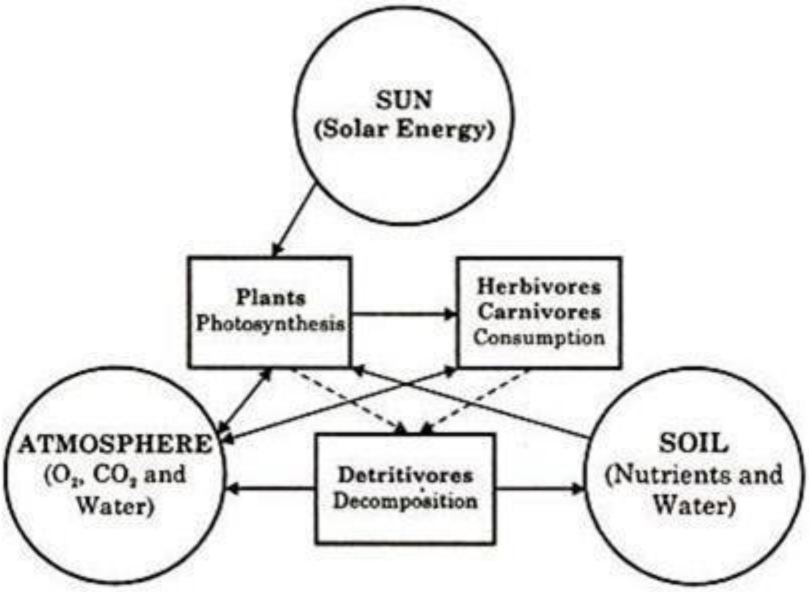
**Structure and Function of an Ecosystem:**

Ecosystem has two main components:

**(1) Abiotic**

**(2) Biotic**


Ecosystem
Abiotic Components
Biotic Components
Climatic
Edaphic
Producers or Autotrophs
Consumers or Heterotrophs
Decomposers or Saptrotrophs
Rain
Light
Wind
Temperature
Soil
pH
Minerals
Topography
Primary Consumers
Secondary Consumers
Tertiary Consumers
Quaternary Consumers




**Relationship within an Ecosystem.**

**(1) Abiotic Components:**

The non living factors or the physical environment existing in an ecosystem form the abiotic components. They have a strong influence on the structure, distribution, behaviour and inter•relationship of organisms.

**Abiotic components are mainly of two types:**

**(a) Climatic Factors:**

Which include rain, temperature, light, wind, humidity etc.

**(b) Edaphic Factors:**

Which include soil, pH, topography, minerals etc.

**The functions of important factors in abiotic components:**

Soils are much more complex than simple sediments. They contain a mixture of weathered rock fragments, highly altered soil mineral particles, organic matter, and living organisms. Soils provide nutrients, water, a home, and a structural growing medium for organisms. The vegetation found growing on top of a soil is closely linked to this component of an ecosystem through nutrient cycling.

The atmosphere provides organisms found within ecosystems with carbon dioxide for photosynthesis and oxygen for respiration. The processes of evaporation, transpiration and precipitation cycle water between the atmosphere and the Earth's surface.

Solar radiation is used in ecosystems to heat the atmosphere and to evaporate and transpire water into

the atmosphere. Sunlight is also necessary for photosynthesis. Photosynthesis provides the energy for plant growth and metabolism, and the organic food for other forms of life.

Most living tissue is composed of a very high percentage of water, up to and even exceeding 90%. The protoplasm of a very few cells can survive if their water content drops below 10%, and most are killed if it is less than 30•50%.

Water is the medium by which mineral nutrients enter and are translocated in plants. It is also necessary for the maintenance of leaf turgidity and is required for photosynthetic chemical reactions. Plants and animals receive their water from the Earth's surface and soil. The original source of this water is precipitation from the atmosphere.

**(2) Biotic Components:**

The living organisms including plants, animals and micro•organisms (Bacteria and Fungi) that are present in an ecosystem form the biotic components.

On the basis of their role in the ecosystem the biotic components can be classified into three main groups:

**(A) Producers**

**(B) Consumers**

**(C) Decomposers or Reducers.**

**(A) Producers:**

The green plants have chlorophyll with the help of which they trap solar energy and change it into chemical energy of carbohydrates using simple inorganic compounds namely water and carbon dioxide. This process is known as photosynthesis. As the green plants manufacture their own food they are known as Autotrophs (i.e. auto = self, trophos = feeder)

The chemical energy stored by the producers is utilised partly by the producers for their own growth and survival and the remaining is stored in the plant parts for their future use.

**(B) Consumers:**

The animals lack chlorophyll and are unable to synthesise their own food. Therefore, they depend on the producers for their food. They are known as heterotrophs (i.e. heteros = other, trophos = feeder)

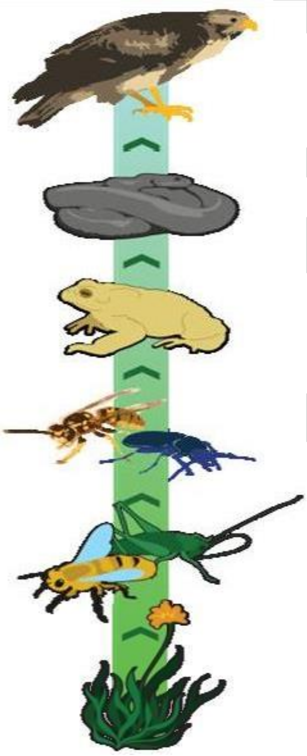
**The consumers are of four types, namely:**

**(a) Primary Consumers**

Primary consumers only eat plants, so they are called herbivores. Eg. Bee and grasshopper.

**(b) Secondary Consumers**

Secondary consumers eat primary consumers; they are carnivores (meat•eaters). Many secondary consumers also eat plants, which makes them omnivores (meat and plant eaters). Eg. Wasp and beetle.



**(c) Tertiary Consumersiary consumers eat the secondary consumers and are usually carnivores (meat eaters). Eg. frog and snake.Decomposers or Reducers:Bacteria and fungi belong to this category. They breakdown the dead organic materials of producers (plants) and consumers (animals) for their food and release to the environment the simple inorganic and organic substances produced as by•products of their metabolisms.These simple substances are reused by the producers resulting in a cyclic ex•change of materials between the biotic community and the abiotic environment of the ecosystem. The decomposers are known as Saprotrophs (i.e., sapros = rotten, trophos = feeder)**

**(d) Quaternary Consumers**

Quaternary consumers eat the tertiary consumers and are carnivores. Eg. Hawk.

**Terrestrial Environment**

Soils are dynamic and develop over time. This process may take decades and even centuries; the organic matter in soils can be thousands of years old. Because of changes in plant growth, temperature, rainfall, disturbance and erosion, a soil that has taken hundreds of years to form can be quickly degraded if

the microbial community is activated. For example, this can occur when a bog soil is drained, which increases oxygen access to the accumulated organic matter. In most soils the major producers of organic matter are the vascular plants, although algae, cyanobacteria, and photosynthetic bacteria also can contribute to these processes, especially in desert crust environments. Soil is the habitat for a variety of organisms, including bacteria, fungi, protozoa, insects, nematodes, worms, and many other animals. Viruses also are present in soils. This complex biological community contributes to the formation, maintenance, and in some situations, the degradation and disappearance of soils.

**Soil as an Environment for Microorganisms**

Soils have been formed, and continue to be formed, in a wide variety of environments. These environments range from Arctic tundra regions, where approximately 11% of the world's soil car- bon pool is stored, to Antarctic dry valleys, where there are no vascular plants. In addition, deeper subsurface zones, where plant roots and their products cannot penetrate, also have micro• bial communities. Microbial activities in these environments can lead to the formation of minerals such as dolomite; microbial activity also occurs in deep continental oil reservoirs, in stones, and even in rocky outcrops. These microbes are dependent on energy sources from algae, nutrients in rainfall, and dust.

Most soils are dominated by inorganic geological materials, which are modified by the biotic community, including the microorganisms and plants, to form soils. A major characteristic of soils is that they are typically not water saturated, making it possible for oxygen to penetrate into the passages and pores. Soils also can contain isolated regions which are water saturated, and these isolated regions become "mini" aquatic environments.

An important defining characteristic of a soil, from a microbial viewpoint, is that the microorganisms are in close physical contact with oxygen; they are located in thin water films on the particle surfaces where oxygen is present at high levels and can be easily replenished from the gaseous phase. When microorganisms use oxygen, it can be replenished rapidly by diffusion, thus maintaining the microbes under aerobic conditions. Oxygen dif• fusion through air in the soil occurs about 4,000 times faster than it does through water. As shown in this figure, the oxygen concentrations and flux rates in pores and channels is high, whereas within water•filled zones the oxygen flux rate is much lower. As an example, particles as small as about 2.0 mm can be aerobic on the outside and anaerobic on the inside. Even in oxygen•saturated zones, microbial•scale aquatic environments can be created that are "hot spots" for anaerobic processes.

Depending on the physical characteristics of the soil, rainfall or irrigation may rapidly change a soil from being ideal, in terms of having reoxygenating thin water films, to an environment with isolated pockets of water, which are "miniaquatic" habitats. If this process of flooding continues, a water-logged soil can be created that is more like a lake sediment.

Shifts in water content and gas fluxes also affect the concentrations of $CO_2$, CO, and other gases present in the soil atmosphere, as noted in**.** These changes will be accentuated in the smaller pores where many bacteria are found. At lower depths less oxygen is available, especially in wetter, less permeable soils. Another factor that affects the levels of oxygen and $CO_2$ in a soil are plant roots. The roots of plants growing in normal aerated soils also consume oxygen and release $CO_2$, influencing the concentrations of these gases in the root environment.

**Microorganisms in the Soil Environment**

The bacteria and fungi use different functional strategies to take advantage of this complex physical matrix. Most soil bacteria are located on the surfaces of soil particles and require water and nutrients that must be located in their immediate vicinity. Bacteria are found most frequently on surfaces within smaller soil pores (2 to 6 μm in diameter). Here they are probably less liable to be eaten by protozoa, unlike bacteria that are located on the exposed outer surface of a sand grain or organic matter particle.Terrestrial filamentous fungi bridge across open areas between soil particles or aggregates called peds, and are exposed to high levels of oxygen. These fungi will tend to darken and form oxygen•impermeable structures including sclerotia and hyphal cords. This is particularly important for the func• tioning of basidiomycetes, which form such structures as an oxy• gen sealing mechanism. Within these structures, the filamentous fungi move nutrients and water over great distances, including across air spaces, a unique part of their functional strategy. These oxidatively polymerized, oxygen•impermeable hyphal bound aries do not usually occur in fungi growing in aquatic environments.

A wide variety of insects and animals also are present in soils, and these often use the fungi and bacteria as food sources, as well as processing plant residues. The earthworms, with their ability to mix and ingest soils, add bacteria and enzymes from their intestine to their soil casts, a process that has major effects on the soil structure and the soil microbial community. Earth•worms, along with many other soil insects and animals, use filamentous fungi as a food source, and this can result in decreased fungal development in a soil. Earthworms also assist in mixing soil organic materials, creating the deep soils found in grasslands. In forest areas, which lack as many earthworms, a more distinct organic matter layer will be formed that is separated from the underlying inorganic layer.

The microbial populations in soils can be very high. In a surface soil the bacterial population can approach $10^8$ to $10^9$ cells per gram dry weight of soil as measured microscopically. Fungi can be present at up to several hundred meters of hyphae per gram of soil. We tend to think that soil fungi are small like the mushrooms sprouting from our lawns. This is natural because most of the fungal thallus lies beneath the soil surface, but such a view sometimes is quite inaccurate. A case in point is the fungus *Armillaria bulbosa*, which lives associated with tree roots in hardwood forests. An individual *Armillaria* clone that covers about 30 acres has been discovered in the Upper Peninsula of Michigan. It is estimated to weigh a minimum of 100 tons (an adult blue whale may weigh 150 tons) and be at least 1,500 years old. Thus some fungal mycelia are among the largest and most ancient living organisms on Earth.

It is important to remember, when discussing soils and their microorganisms, that only a minor portion (approximately 10%) of the microscopically observable organisms making up this biomass have been cultured. In terms of biotechnology and basic ecology, the microorganisms that have not been cultured may provide a valuable genetic resource for basic and applied research. We are gradually learning more about these uncultured microorganisms through the use of molecular techniques. For example, novel groups of the *Crenarchaeota* have been discovered in forest soil and the ocean by extracting microbial DNA and amplifying it with the polymerase chain reaction. Examination of soils from different areas of the world continues to yield surprises. Recent molecular studies of Siberian tundra soils have uncovered considerable unexplored microbial diversity, as most of the recovered bacteria are not related to any known species. Microorganisms are present and prolific in subsurface environments, including oil reservoirs. Hyperthermophilic archaea have been found in such harsh subsurface environments and are probably indigenous to these poorly understood regions of our world.

The gram•positive bacteria, which show varied degrees of branching and mycelial development, are an important and less studied part of the soil microbial community. They include the coryneforms, the nocardioforms, and the true filamentous bacteria or actinomycetes. These bacteria play a major role in the degradation of hydrocarbons, older plant materials, and soil humus. In addition, some members of these groups actively degrade pesticides. The filamentous actinomycetes, primarily of the genus *Streptomyces*, produce an odor•causing compound called geosmin, which gives soils their characteristic earthy odor.

As with aquatic environment, microorganisms are constantly being added to soils from waters, wind, dust, plants, and animal sources. Most of these added microorganisms will not survive, either being outcompeted by indigenous microbes or being consumed by predators such as the protozoa.

The microbial community in soil makes important contributions to biogeochemical cycling and the carbon, nitrogen, sulfur, iron, and manganese cycles. Because the soil is primarily an oxidized environment, the inorganic forms of these elements will tend to be in the oxidized state. If there are localized water•saturated, lower oxygen•flux environments, the biogeochemical cycles will shift toward reduced species.

Soil microorganisms can be categorized with respect to organic matter processing on the basis of both (1) their preference for either easily available or more resistant substrates and (2) the substrate concentrations they require. At higher nutrient•level levels, some microorganisms such as *Pseudomonas* respond rapidly to the addition of easily usable substrates such as sugars and amino acids. Indigenous forms tend to use native organic matter to a greater extent. These include the genus *Arthrobacter* and many soil actinomycetes. A less understood part of the microbial community grows in oligotrophic environments, defined as those that contain less than 15 mg/liter of organic matter. Physiological and genetic studies on microbial responses in such low•nutrient environments are under way. As discussed in this book, microbes have many strategies to deal with survival in such low•nutrient environments, and this presents special technical problems when studying them.

**Microorganisms and the Formation of Different Soils**

Soils form under various environmental conditions. When newly exposed geologic materials begin to weather, as after a volcanic event or a simple soil disturbance, microbial colonization occurs. If only subsurface materials are available, phosphorus may be present, but nitrogen and carbon must be imported by physical or biological processes. Under these circumstances many cyanobacteria, which can fix atmospheric nitrogen and carbon, are active in pioneer•stage nutrient accumulation.

Once they are formed, most soils are rich sources of nutrients. Nutrients are found in organic matter, microorganisms, soil insects, and other animals. Plants grow, senesce, and die—and at each of these phases, they provide nutrients for soil organisms. Different plant parts vary in their nutrient content and biomasses. In addition, the turnover times for the various plant parts are quite different. The components in the plant•soil system with the lowest carbon•nitrogen ratios (most nutrient•rich) are soil organic matter, microorganisms, soil insects, and other soil animals. The soil organic matter contains the greatest portion of the carbon and nitrogen in a typical soil, but with its slow turnover time (100 to 1,000 years and longer), most of this nutrient resource is not immediately available for plant or microbial use.

**Tropical and Temperate Region Soils**

In moist tropical soils, with their higher mean temperatures, organic matter is decomposed very quickly, and the mobile inorganic nutrients can be leached out of the surface soil environment, causing a rapid loss of fertility. To limit nutrient loss, many tropical plants have root systems that penetrate the rapidly decomposing litter layer. As soon as organic material and minerals are released during decomposition, the roots can take them up to avoid losses in leaching. Thus it is possible to "recycle" nutrients before they are lost with water movement through the soil (figure 30.4*a*). With deforestation, the nutrients are not recycled, leading to their loss from the soil and decreased soil fertility.

Tropical plant•soil communities are often used in slash•and• burn agriculture. The vegetation on a site is chopped down and burned to release the trapped nutrients. For a few years, until the minerals are washed from the soils, crops can be grown. When the minerals are lost from these low organic matter soils, the farmer must move to a new area and start over by again cutting and burning the native plant community. This cycle of slash•and•burn agri• culture is stable if there is sufficient time for the plant community to regenerate before it is again cut and burned. If the cycle is too short, as can occur with overpopulation, rapid and almost irreversible

degradation of the soil can occur.

In many temperate region soils, in contrast, the decomposition rates are less than that of primary production, leading to litter accumulation. Deep root penetration in temperate grasslands results in the formation of fertile soils, which provide a valuable resource for the growth of crops in intensive agriculture.

The soils in many cooler coniferous forest environments suffer from an excessive accumulation of organic matter as plant litter. In winter, when moisture is available, the soils are cool, and this limits decomposition. In summer, when the soils are warm, water is not as available for decomposition. Organic acids are produced in the cool, moist litter layer, and they leach into the underlying soil. These acids solubilize soil components such as aluminum and iron, and a bleached zone may form. Litter continues to accumulate, and fire becomes the major means by which nutrient cycling is maintained. Controlled burns are becoming a more important part of environmental management in this type of plant•soil system.

Bog soils provide a unique set of conditions for microbial growth. In these soils the decomposition rate is slowed by the waterlogged, predominantly anoxic conditions, which lead to peat accumulation. When such areas are drained, they become more aerobic and the soil organic matter is degraded, resulting in soil subsidence. Under aerobic conditions the lignin•cellulose complexes of the accumulated organic matter are more susceptible to decomposition by the filamentous fungi.

**Cold Moist Area Soils**

Soils in cold environments, whether in Arctic, Antarctic, or alpine regions, are of extreme interest because of their wide distribution and impacts on global•level processes. The colder mean soil temperatures at these sites decrease the rates of both decomposition and plant growth. In these cases soil organic matter accumulates, and plant growth can become limited due to the immobilization of nutrients in soil organic matter. Often, below the plant growth zone, these soils are permanently frozen. These permafrost soils hold 11% of the Earth's soil carbon and 95% of its organically bound nutrients. These soils are very sensitive to physical disturbance and pollution, and the widespread exploration of such areas for oil and minerals can have long•term effects on their structure and function. When plants are covered by snow, plant diseases are caused by snow molds, which can grow and attack plants at —5°C. These fungi produce special antifreeze proteins (AFP) that enable them to function under these harsh conditions. In water•saturated bog areas, bacteria are more important than fungi in decomposition processes, and there is decreased degradation of lignified materials. As in other soils, the nutrient cycling processes of nitrification, denitrification, nitrogen fixation, and methane synthesis and utilization, although occurring at slower rates, can have major impacts on global gaseous cycles.

**Desert soils**

Soils of hot and cold arid and semiarid deserts are dependent on periodic and infrequent rainfall. When these rainfalls occur, water can puddle in low areas and be retained on the soil surface by microbial communities called desert crusts. These consist of cyanobacteria and associated commensalistic microbes, including *Anabaena, Microcoleus, Nostoc,* and *Scytonema.* The depth of the photosynthetic layer is perhaps 1 mm, and the cyanobacterial filaments and slimes link the sand particles, which change the surface soil albedo, water infiltration rate, and susceptibility to erosion. These crusts are quite fragile, and vehicle damage can be evident for decades. After a rainfall, nitrogen fixation will begin within approximately 25 to 30 hours, and when the rain has evaporated or drained into the soil, the crust will dry up and nitrogen will be released for use by other microorganisms and the plant community.

**Geologically Heated Hyperthermal Soils**

Geologically heated soils are found in such areas as Iceland and the Kamchatka peninsula in eastern

Russia, and such heated soils also occur at many mining waste sites. An important microorganism found in heated mining wastes is *Thermoplasma.* These soils are populated by bacterial and archaeal procaryotes, many of which are chemolithoautotrophs. A wide variety of chemoorganotrophic genera also are found in these environments; these include the aerobes *Thermomicrobium, Thermoleophilum,* and also the anaerobes *Thermosipho* and *Thermo toga.* Such geothermal soils have been of great interest as a source of new microbes to use in biotechnology, and the search for new, unique microorganisms in these areas is intensifying all over the world.

**Aquatic ecosystem**

An **aquatic ecosystem** is an ecosystem in a body of water. Communities of organisms that are dependent on each other and on their environment live in aquatic ecosystems. The two main types of aquatic ecosystems are marine ecosystems and freshwater ecosystems.

Types

**Marine**

Marine ecosystems cover approximately 71% of the Earth's surface and contain approximately 97% of the planet's water. They generate 32% of the world's net primary production. They are distinguished from freshwater ecosystems by the presence of dissolved compounds, especially salts, in the water. Approximately 85% of the dissolved materials in seawater are sodium and chlorine. Seawater has an average salinity of 35 parts per thousand (ppt) of water. Actual salinity varies among different marine ecosystems.

Marine ecosystems can be divided into many zones depending upon water depth and shoreline features. The oceaniczone is the vast open part of the ocean where animals such as whales, sharks, and tuna live. The benthic zone consists of substrates below water where many invertebrates live. The intertidal zone is the area between high and low tides; in this figure it is termed the littoral zone. Other near•shore (neritic) zones can include estuaries, salt marshes, coral reefs,lagoons and mangrove swamps. In the deep water, hydrothermal vents may occur where chemosynthetic sulfur bacteriaform the base of the food web.

Classes of organisms found in marine ecosystems include brown algae, dinoflagellates, corals, cephalopods,echinoderms, and sharks. Fishes caught in marine ecosystems are the biggest source of commercial foods obtained from wild populations.

Environmental problems concerning marine ecosystems include unsustainable exploitation of marine resources (for example overfishing of certain species), marine pollution, climate change, and building on coastal areas.

**Freshwater**

Freshwater ecosystems cover 0.78% of the Earth's surface and inhabit 0.009% of its total water. They generate nearly 3% of its net primary production. Freshwater ecosystems contain 41% of the world's known fish species.

There are three basic types of freshwater ecosystems:

Lentic: slow moving water, including pools, ponds, and lakes.

Lotic: faster moving water, for example streams and rivers.

Wetlands: areas where the soil is saturated or inundated for at least part of the time.

**Lentic**

Lake ecosystems can be divided into zones. One common system divides lakes into three zones (see figure). The first, the littoral zone, is the shallow zone near the shore. This is where rooted wetland plants occur. The offshore is divided into two further zones, an open water zone and a deep water zone. In the open water zone (or photic zone) sunlight supports photosynthetic algae, and the species that feed upon them. In the deep water zone, sunlight is not available and the food web is based on detritus entering from the littoral and photic zones. Some systems use other names. The off shore areas may be called thepelagic zone, the photic zone may be called the limnetic zone and the aphotic zone may be called theprofundal zone. Inland from the littoral zone one can also frequently identify a riparian zone which has plants still affected by the presence of the lake—this can include effects from windfalls, spring flooding, and winter ice damage. The production of the lake as a whole is the result of production from plants growing in the littoral zone, combined with production from plankton growing in the open water.

Wetlands can be part of the lentic system, as they form naturally along most lake shores, the width of the wetland and littoral zone being dependent upon the slope of the shoreline and the amount of natural change in water levels, within and among years. Often dead trees accumulate in this zone, either from windfalls on the

shore or logs transported to the site during floods. This woody debris provides important habitat for fish and nesting birds, as well as protecting shorelines from erosion.

Two important subclasses of lakes are ponds, which typically are small lakes that intergrade with wetlands, and water reservoirs. Over long periods of time, lakes, or bays within them, may gradually become enriched by nutrients and slowly fill in with organic sediments, a process called succession. When humans use the watershed, the volumes of sediment entering the lake can accelerate this process. The addition of sediments and nutrients to a lake is known as eutrophication

**Ponds**

Ponds are small bodies of freshwater with shallow and still water, marsh, and aquatic plants.[5] They can be further divided into four zones: vegetation zone, open water, bottom mud and surface film.[6] The size and depth of ponds often varies greatly with the time of year; many ponds are produced by spring flooding from rivers. Food websare based both on free•floating algae and upon aquatic plants. There is usually a diverse array of aquatic life, with a few examples including algae, snails, fish, beetles, water bugs, frogs, turtles, otters and muskrats. Top predators may include large fish, herons, or alligators. Since fish are a major predator upon amphibian larvae, ponds that dry up each year, thereby killing resident fish, provide important refugia foramphibian breeding.[7] Ponds that dry up completely each year are often known as vernal pools. Some ponds are produced by animal activity, including alligator holes and beaver ponds, and these add important diversity to landscapes.[7]

**Lotic**

The major zones in river ecosystems are determined by the river bed's gradient or by the velocity of the current. Faster moving turbulent water typically contains greater concentrations of dissolved oxygen, which supports greater biodiversity than the slow moving water of pools. These distinctions form the basis for the division of rivers intoupland and lowland rivers. The food base of streams within riparian forests is mostly derived from the trees, but wider streams and those that lack a canopy derive the majority of their food base from algae. Anadromous fish are also an important source of nutrients. Environmentalthreats to rivers include loss of water, dams, chemical pollution and introduced species. A dam produces negative effects that continue down the watershed. The most important negative effects are the reduction of spring flooding, which damages wetlands, and the retention of sediment, which leads to loss of deltaic wetlands.

**Wetlands**

Wetlands are dominated by vascular plants that have adapted to saturated soil.[7] There are four main types of wetlands: swamp, marsh, fen and bog (both fens and bogs are types of mire). Wetlands are the most productive natural ecosystems in the world because of the proximity of water and soil. Hence they support large numbers of plant and animal species. Due to their productivity, wetlands are often converted into dry land with dykes and drains and used for agricultural purposes. The construction of dykes, and dams, has negative

consequences for individual wetlands and entire watersheds. Their closeness to lakes and rivers means that they are often developed for human settlement. Once settlements are constructed and protected by dykes, the settlements then become vulnerable to land subsidence and ever increasing risk of flooding. The Louisiana coast around New Orleans is a well•known example; the Danube Delta in Europe is another.

**Functions**

Aquatic ecosystems perform many important environmental functions. For example, they recycle nutrients, purify water, attenuate floods, recharge ground water and provide habitats for wildlife. Aquatic ecosystems are also used for human recreation, and are very important to the tourism industry, especially in coastal regions. The health of an aquatic ecosystem is degraded when the ecosystem's ability to absorb a stress has been exceeded. A stress on an aquatic ecosystem can be a result of physical, chemical or biological alterations of the environment. Physical alterations include changes in water temperature, water flow and light availability. Chemical alterations include changes in the loading rates of biostimulatory nutrients, oxygen consuming materials, and toxins. Biological alterations include over•harvesting of commercial species and the introduction of exotic species. Human populations can impose excessive stresses on aquatic ecosystems. There are many examples of excessive stresses with negative consequences. Consider three. The environmental history of the Great Lakes of North America illustrates this problem, particularly how multiple stresses, such as water pollution, over•harvesting and invasive species can combine.[11] The Norfolk Broadlands in England illustrate similar decline with pollution and invasive species.[12] Lake Pontchartrain along the Gulf of Mexico illustrates the negative effects of different stresses including levee construction, logging of swamps, invasive species and salt water intrusion.

**Abiotic characteristics**

An ecosystem is composed of biotic communities that are structured by biological interactions and abiotic environmental factors. Some of the important abiotic environmental factors of aquatic ecosystemsinclude substrate type, water depth, nutrient levels, temperature, salinity, and flow.[7][10] It is often difficult to determine the relative importance of these factors without rather large experiments. There may be complicated feedback loops. For example, sediment may determine the presence of aquatic plants, but aquatic plants may also trap sediment, and add to the sediment through peat.

The amount of dissolved oxygen in a water body is frequently the key substance in determining the extent and kinds of organic life in the water body. Fish need dissolved oxygen to survive, although their tolerance to low oxygen varies among species; in extreme cases of low oxygen some fish even resort to air gulping. Plants often have to produce aerenchyma, while the shape and size of leaves may also be altered. Conversely, oxygen is

fatal to many kinds of anaerobic bacteria.

Nutrient levels are important in controlling the abundance of many species of algae. The relative abundance of nitrogen and phosphorus can in effect determine which species of algae come to dominate. Algae are a very important source of food for aquatic life, but at the same time, if they become over•abundant, they can cause declines in fish when they decay. Similar over•abundance of algae in coastal environments such as the Gulf of Mexico produces, upon decay, a hypoxic region of water known as a dead zone.

The salinity of the water body is also a determining factor in the kinds of species found in the water body. Organisms in marine ecosystems tolerate salinity, while many freshwater organisms are intolerant of salt. The degree of salinity in an estuary or delta is an important control upon the type of wetland (fresh, intermediate, or brackish), and the associated animal species. Dams built upstream may reduce spring flooding, and reduce sediment accretion, and may therefore lead to saltwater intrusion in coastal wetlands.

Freshwater used for irrigation purposes often absorbs levels of salt that are harmful to freshwater organisms.

**Biotic characteristics**

The biotic characteristics are mainly determined by the organisms that occur. For example, wetland plants may produce dense canopies that cover large areas of sediment—or snails or geese may graze the vegetation leaving large mud flats. Aquatic environments have relatively low oxygen levels, forcing adaptation by the organisms found there. For example, many wetland plants must produce aerenchyma to carry oxygen to roots. Other biotic characteristics are more subtle and difficult to measure, such as the relative importance of competition, mutualism or predation. There are a growing number of cases where predation by coastal herbivores including snails, geese and mammals appears to be a dominant biotic factor.

**Autotrophic organisms**

Autotrophic organisms are producers that generate organic compounds from inorganic material. Algae use solar energy to generate biomass from carbon dioxide and are possibly the most important autotrophic organisms in aquatic environments.[16] Of course, the more shallow the water, the greater the biomass contribution from rooted and floating vascular plants. These two sources combine to produce the extraordinary production of estuaries and wetlands, as this autotrophic biomass is converted into fish, birds, amphibians and other aquatic species.

Chemosynthetic bacteria are found in benthic marine ecosystems. These organisms are able to feed on hydrogen sulfide in water that comes from volcanic vents. Great concentrations of animals that feed on these bacteria are found around volcanic vents. For example, there are giant tube worms (*Riftia pachyptila*) 1.5 m in length and clams (*Calyptogena magnifica*) 30 cm long.

**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 ownbiomass.[16] Euryhaline organisms are salt tolerant and can survive in marine ecosystems, while stenohaline or salt intolerant species can only live in freshwater environments.

**MICROBIOLOGY OF AIR**

**Introduction:**

Of all environments, air is the simplest one and it occurs in a single phase gas. Various layers can be recognized in the atmosphere up to a height of about 1000km. The layer nearest to the earth is called as troposphere. This troposphere is characterized by a heavy load of microorganisms. The atmosphere as a habitat is characterized by high light intensities, extreme temperature variations, low amount of organic matter and a scarcity of available water making it a non hospitable environment for microorganisms and generally unsuitable habitat for their growth. Nevertheless, substantial numbers of microbes are found in the lower regions of the atmosphere. The study of these microbes in air is called as Aero Microbiology. With respect to environmentalist Microbiology is the study of the composition and physiology of microbial communities in the environment i.e. the soil, water, air and sediments covering the planet. Can also include the microorganisms living on or in the animals and plants that inhabit these areas

**Disease caused by air borne microbes:**

**Bacterial Diseases**

**Brucellosis:**

Brucella suis it is mainly an occupational disease among veterinarian, butcher and slaughter house workers.

**Pulmonary Anthrax:**

Bacillus anthracis is the causative agent. Transmission is mainly by inhaling the dust contaminated by animal products.

**Diseases Caused by Streptococcus Pyogenes:**

A number of diseases are caused by Streptococcus pyogenes which is mainly transmitted through air. Diseases Caused by Streptococcus pyogenes occur in the throat, skin, and systemically.

**Rheumatic Fever:**

This is upper respiratory tract infection by S. pyogenes Characterized by inflammation and degeneration of heart valves.

**Streptococcal Pneumonia:**

It is of major occurrence among the bacterial pneumonia. Causative agent is

**Streptococcus pneumonia**

**Meningitis :**

Haemophilus influenzae causes meningitis in children between 6 weeks and 2 years of age.

**Diptheria:**

Diphtheria is mainly contracted by children. Infection of the tonsils, throat and nose and generalized toxemia are the symptoms. The causative agent is

Corynebacterium diphtheria

**Tuberculosis:**

Pulmonary tuberculosis is a severe respiratory disease. Loss of appetite, fatigue, weight loss, night sweats and persistent cough are some of the symptoms. Causative agent is Mycobacterium tuberculosis

**Legionellosis:**

It is a type of branchopneumonia. Legionella pneumophila is the causative agent. It occurs in natural water. At times it enters and proliferates in cooling tower, air cooler and shower bath. Spraying and splashing of water containing pathogen may produce aerosols which are disseminated in air.

**Air Borne Fungal Diseases:**

**It consists of many types. They are following**

**Cryptococcosis:**

Leads to mild pneumonitis. Causative agent is the yeast Cryptococcus neoformans. It is a soil saprophyte. Infection is acquired by inhalation of soil particles containing the causative agent.

**Blastomycosis:**

Formation of suppurative and granulomatous lesions in any part of the body.

Blastomyces dermatitis is the causative agent. It is a soil borne fungus and hence inhalation of soil particles containing the fungus produces the infection.

**Coccidiodomycosis:**

Infection may not be apparent but in severe cases it is fatal. Usually infection leads to self•limited influenza fever known as valley fever or desert rheumatism. Causative agent of the disease is a soil fungus, Coccidioides immitis. Inhalation of dust containing arthrospores of the fungus leads to infection.

**Aspergillosis:**

It is an opportunistic disease of human. Causative agent is Aspergillus fumigatus. Infection occurs through inhalation of spores.

**Air Borne Viral Diseases:**

Air borne viral diseases are of different types. They are following,

**Common Cold:**

It is the most frequent of all human infections. Characteristic symptom includes running noses. Rhinovirus is the causative agent. Droplets with nose and throat discharges from infected persons are the source.

**Influenza:**

Symptoms of influenza are nasal discharge, head ache, muscle pains, sore throat and general weakness. Causative agents are orthomyxovirus.

**Measles:**

Measles is the most common communicable human disease mainly affecting children. Symptoms are fever, cough, and cold and red, blotchy skin rash. Causative virus is morbillivirus. Source of infection is respiratory tract secretions in the form of droplets.

**Mumps:**

It is a communicable disease and is a common childhood disease. It is characterized by painful swelling of parotid glands and salivary glands. Mumps virus causes the disease. Droplets containing infected saliva are the main source.

**Adeno Viral Diseases:**

Adenoviruses cause acute self•limiting respiratory and eye infections. Adenoviruses are transmitted by

airborne mode. Diseases include acute febrile pharyngitis, acute respiratory disease and adenovirus pneumonia.

**SOURCES OF MICROORGANISM IN AIR:**

Although a number of microorganisms are present in air, it doesn't have an indigenous flora. Air is not a natural environment for microorganisms as it doesn't contain enough moisture and nutrients to support their growth and reproduction.

Quite a number of sources have been studied in this connection and almost all of them have been found to be responsible for the air micro flora. One of the most common sources of air micro flora is the soil. Soil microorganisms when disturbed by the wind blow, liberated into the air and remain suspended there for a long period of time. Man made actions like digging or ploughing the soil may also release soil borne microbes into the air. Similarly microorganisms found in water may also be released into the air in the form of water droplets or aerosols. Splashing of water by wind action or tidal action may also produce droplets or aerosols. Air currents may bring the microorganisms from plant or animal surfaces into air. These organisms may be either commensals or plant or animal pathogens. Studies show that plant pathogenic microorganisms are spread over very long distances through air. For example, spores of Puccini a graminis travel over a thousand kilometers. However, the transmission of animal diseases is not usually important in out side air.

The main source of airborne microorganisms is human beings. Their surface flora may be shed at times and may be disseminated into the air. Similarly, the commensal as well as pathogenic flora of the upper respiratory tract and the mouth are constantly discharged into the air by activities like coughing, sneezing, talking and laughing. The microorganisms are discharged out in three different forms which are grouped on the basis of their relative size and moisture content. They are droplets, droplet nuclei and infectious dust. It was Wells, who described the formation of droplet nuclei. This initiated the studies on the significance ofairborne transmission.

**Droplet:**

Droplets are usually formed by sneezing, coughing or talking. Each consists of saliva and mucus. Droplets may also contain hundreds of microorganisms which may be pathogenic if discharged from diseased persons. Pathogens will be mostly of respiratory tract origin. The size of the droplet determines the time period during which they can remain suspended. Most droplets are relatively large, and they tend to settle rapidly in still air. When inhaled these droplets are trapped on the moist surfaces of the respiratory tract. Thus, the droplets containing pathogenic microorganisms may be a source of infectious disease.

**Droplet Nuclei:**

Small droplets in a warm, dry atmosphere tend to evaporate rapidly and become droplet nuclei. Thus, the residue of solid material left after drying up of a droplet is known as droplet nuclei. These are small, 1•4µm,

and light. They can remain suspended in air for hours or days, traveling long distances. They may serve as a continuing source of infection if the bacteria remain viable when dry. Viability is determined by a set of complex factors including, the atmospheric conditions like humidity, sunlight and temperature, the size of the particles bearing the organisms, and the degree of susceptibility or resistance of the particular microbial species to the new physical environment. If inhaled droplet nuclei tend to escape the mechanical traps of the upper respiratory tract and enter the lungs. Thus, droplet nuclei may act as more potential agents of infectious diseases than droplets. Droplets are usually formed by sneezing, coughing and talking. Each droplet consists of saliva and mucus and each may contain thousands of microbes. It has been estimated that the number of bacteria in a single sneeze may be between 10,000 and 100,000. Small droplets in a warm, dry atmosphere are dry before they reach the floor and thus quickly become droplet nuclei.

**Infectious Dust:**

Large aerosol droplets settle out rapidly from air on to various surfaces and get dried. Nasal and throat discharges from a patient can also contaminate surfaces and become dry. Disturbance of this dried material by bed making, handling a handkerchief having dried secretions or sweeping floors in the patient's room can generate dust particles which add microorganisms to the circulating air. Most dust particles laden with microorganisms are relatively large and tend to settle rapidly. Droplets expelled during coughing, sneezing, etc
consist of sativa and mucus, and each of them may contain thousands of microorganisms. Most droplets are

large, and, like dust, tend to settle rapidly. Some droplets are of such size that complete evaporation. Occurs in a warm, dry climate, and before they reach the floor quickly become droplet nuclei. These are small and light, and may float about for a relatively long period. Airborne diseases are transmitted by two types of droplets, depending upon their size.

(1) Droplet infection proper applies to, droplets larger than 100 µm in diameter.

**(2)** The other type may be called airborne infection, and applies to dried residues of droplets. Droplet infection remains localized and concentrated, whereas airborne infection may be carried long distances arid is dilute. Microorganisms can survive for relatively longer periods in dust. This creates a significant hazard, especially in hospital areas. Infective dust can also be produced during laboratory practices like opening the containers of freeze dried cultures or withdrawal of cotton plugs that have dried after being wetted by culture fluids. These pose a threat to the people working in laboratories.

**MICROBES IN ATMOSPHERE:**

The atmospheric layers and the airflow pattern are the important forces in determining the distribution and dynamics of viable particles in air. The aero microbiological pathway (AMP) involves the path and pattern of movement of microbial particles in atmosphere. The layer of most interest and significance in aero microbiological is the boundary layer, which extends up to 0.1km form the earth's surface. However, that airborne transport of microorganisms is by no means limited to this layer and it is not uncommon to have microorganisms associated with layers of the troposphere above the turbulent boundary layer. However, it is the surface boundary layer that is largely responsible for the transport of particles over both short and long distances. The boundary layer consist of three parts: the laminar boundary layer, the turbulent boundary layer and the local eddy layer.

The laminar boundary layer is the layer of still air associated with the earth and all projecting surfaces, weather solid or liquid. This layer can be any where from 1 µm to several meters thick depending upon weather conditions. Still condition cause the thickness of this layer to increase and windy conditions minimize it to a very thin layer that remains in close association with surfaces. The turbulent boundary layer is the layer that is considered to be always in motion and responsible for horizontal transport phenomena (wind dispersion), which occurs whenever micro•organisms associated particles are launched either indoors or outdoors. In the lower level of turbulent layer, the linear flow of air is interrupted by surface projections and their associated laminar boundary layers. The interaction results in the formation of friction against the air flow. This friction, which is apparent in the form of local areas of "swirling: turbulence, determines rate of movement of these particles. The local eddy layer is the actual zone of interaction between the still laminar boundary layer of surface projections and the turbulent boundary layer.

**Dispersal of microbes in Atmosphere:**

The dispersal of microbes in air begins with the discharge of microbial cells, spores or particle loaded with viable particles (aerosol) to the atmosphere. It is followed by the subsequent transport via diffusion and dispersion of these particles and finally their deposition on any surface. An example of this pathway is that of liquid aerosols containing the influenza virus launched into the air through cough, sneeze or even through the air, inhaled and deposited in the lungs of a near by person, where they may begin a new infection. Traditionally the deposition of viable microorganisms and the resultant infection are given the most attention, but all three processes (launching, transport and deposition) are of equal importance in understanding the aerobiological pathway. While a microbial particle (hypha, cell or spore) germinate and grow, when dispersed on compatible surface, gaining the metabolic efficiency, it perishes on coming in contact with an incompatible surface.

**Bioaerosol:**

The bioaerosol are the atmospheric particles, mist of dust of µm range, associated with metabolically active or inactive viable particles. Bioaerosols vary considerably in size and composition depends on a variety of factors including the type of microorganism or toxin, the type of particles they are associated with such a mist or dust and gases in which the bioaerosol is suspended. Bioaerosol in general range from 0.02•100 µm in diameter and are classified on the basis of their size. The smaller the particle <0.1 µm in diameter are considered to be in the nuclei mode, those ranging from 0.1•2 µm are in the accumulation mode and the larger particles are considered to be in the coarse mode, which undergo rapid sedimentation. The particles in nuclei or accumulation ode are considered to be fine particles and have the capacity to move long distances. These particles have also a long residence time in the environment. The particle in coarse mode are considered coarse particles a they settle within few meters to few kilometers from the source. The composition of bioaerosol can be liquid or solid or the mixture of the two and should b thought of as microorganism associated with air borne particles containing microorganism. This is because it is rare to have microorganism (toxins) that are not associated with other airborne particles such as dust or mist.

**Launching :**

The process whereby microbes loaded particles become suspended within the earth's atmosphere is termed launching. Because bioaerosol must be launched in to the atmosphere to be transported. The launching of bioaerosol is mainly from terrestrial and aquatic sources, with greater airborne concentrations or atmospheric loading being associated with terrestrial sources than with aquatic sources. The contribution of aerial source is considered minimum. This phenomenon is related to the limited potential for microorganisms to reproduce with airborne. This however an area of aeromicrobiology for which there is little available information is. In addition, a significant contribution of viable particles to the atmosphere is also made from surfaces of plants and animals.

Launching in to surface boundary layers is influenced by a number of factors such as: (a) air turbulence created by the movement of humans, animals and machines; (b) the generation, storage, treatment and disposal of waste material; (c) natural mechanical processes such as the action of water and wind on contaminated solid or liquid surfaces; and (d) the release of fungal spores as a result of natural fungal life cycles. Airborne particles can be launched from either point, linear, or area sources. A point source is an isolated and well defined site of launching such as a pile of biosoild material, before it is applied over the field or an infected leaf of a plant launching the spores of a pathogen to air. Point sources tend to play general conical•type dispersion. Point sources can be further defined on the basis of launching phenomenon: (1) instantaneous point sources, for example, a single event such as a sneeze, or (2) continuous point sources, from which launching occurs over extended period of time, such as the biosolid pile. In contrast to point sources, linear sources and area sources involve larger, less well defined areas. When considered on same size scale, linear and area sources display more particulate wave dispersion as opposed to the conical type of dispersion displayed by point sources. Linear and area sources can also be divided into instantaneous and continuous launching points of origin.For example, an instantaneous linear source might be a passing aircraft releasing a biological warfare agent or a passenger jet releasing the unburnt carbon particles source

**Bio aerosol transport:**

Transport or dispersion is the process by which a viable particle moves from one point to another with the speed of wind or when it is launched in to air with a force. The force of airborne particle is dependent on the kinetic energy gained by it from the force at which it is launches to the atmosphere and the wind speed. Transport of bioaerosols can be defined in terms of time and distance. Submicroscale transport involves short periods of time, under 10 minutes, as well as short distances, under 100m. this type of transport is common within buildings or other confined spaces. Micro transport ranges from 10 minutes to 1 hour and from 100 m to 1 km and is most common type of transport phenomenon

Because most microorganism have limited ability to survive with suspended in atmosphere, the most common scales considered are the submicroscale and micro scale . Some macro scale transport can be global in nature importantly on pathogenic point of view like spores of wheat rust fungus.

As bioaerosol travel through time and space, different forces act upon them such as diffusion, inactivation and ultimately deposition. The relative amount of diffusion that may occur in association with particulates such as bioaerosols can be estimated by using the method of Osbert Reynolds. He said that factors associated with wind could provide an indication of the amount of turbulence associated with linear flow.

The limiting value for the renoylds equation is usually considered to be 2000 (for an object with 5cm diameter, the non turbulent wind speed is 2 kmlhr), with values above this number indicating turbulent conditions. The higher the value, greater the relative turbulence of airflow and micro•organism associated particles diffusion that occurs per unit time. Thus the rate of diffusion and transport is directly proportional to the value of Re.

**Bioaerosol deposition:**

Bioaerosol is regarded as the last step in AMB pathway. Depending on the size and the kinetic energy gained by it during launching and transport. Under standard conditions, however, the rate of deposition of a particle is directly proportional to its mass, volume and mass/volume ratio.

**Gravitational settling**:

The main mechanism associated with deposition is the action of gravity on particles. Force acts on the particles heavier then air, pulling them down. Larger particles will have higher velocities and will settle down of the AMB pathway faster. It should be however noted that for particles of microbiological relevance that are exposed to winds above 8 ×103 m/hr, gravitational deposition may be negligible unless the particles cross out of the laminar flow via processes such as downward molecular diffusion or increase in density because of condensation reaction such as air deposition.

**(a) Downward molecular diffusion:**

It is a randomly occurring process caused by natural air currents eddies that promote and enhance the downward movement of air borne particulate matters. Molecular diffusion is also influenced by the force of the wind and deposition rate increases with increasing wind speed and turbulence of air.

**(b) Surface impaction:**

It is a process in which particles make contact with surfaces, such as leaves, trees, wall and furniture, with impaction there is an associated loss of kinetic energy. In nature, it is rare to find flat, smooth surfaces on which wind currents are unobstructed. Thus, surface impaction is a very critical factor influencing the rate of deposition of aerosols. The impaction potential causes the the collision of a particle to the surface and facilitates its attachment to the same. However, depending on the nature of the surface of a particle can bounce after

collision. Bouncing off a surface causes the particle to reenter the air current at a lower rate, which can have one of the two effects: (1) it can allow subsequent downward molecular diffusion and gravitational settling to occur, resulting in deposition on another nearby surface, or (2) it can allow the particle to escape the surface and once again reenter the air current. Studies have shown that impaction is influenced by the velocity and size of the particle as ell as the size, shape and nature of surface it is approaching.

**D) Rain and electrostatic deposition:**

Rainfall and electrostatic charge can also affect deposition. Rainfall deposition occurs a s a condensation reaction between two particles, which combine and create a bioaerosol with a greater mass, making it to settle

faster. The overall efficiency of rain deposition also depends upon the spread area of the particle plume.
Larger, more diffuse plumes undergo stronger impaction than smaller, more concentrated plumes. Rain deposition is also affected by the intensity of rain fall. Electrostatic deposition also condenses bioaerosols, but it is based on electrovalent particle attraction. All particles tend to have some type of associated charge. Microorganisms typically have an overall negative charge associated with their surfaces at neutrals pH.these negatively charged particles can associate with other positively charged airborne particles, resulting in electrostatic condensation.

**Outdoor aero microbiology:**

In outdoor or extramural environment, the expanse of space and the presence of air turbulence are the two controlling factors in the movement of bioaerosols. Brief account of these areas are given below

**Indoor aero microbiology:**

It involves home and work place environments in which air borne microbes create major public health concerns. Microbes can survive for extended period in indoors as they have relatively less exposure to radiations. Some of the indoor environments are described as following

**Private homes and office building:**

Extent of bioaerosols development determines the health of any building. These include several factors that influence the formation of bioaerosols. this include the presence of air filtering systems designed and fitted in the building , the health and hygiene of the occupants, the amount of clean outdoor air circulated through the building, the type of lightning, the ambient temperature in the building and the relative humidity. In spite of all precautions some microbes may develop mechanism for survival and transmission.

**Hospital and laboratories:**

These two indoor environments have such potential for the aerosolisation of pathogenic microbes. Microbiological laboratories are also a breeding center for pathogenic microbes.

**Space flight:**

Microbes have been detected even from harsh environments. They are associated with every aspect of life even space craft. Microbes are also beneficial for us. Air purification is an example of a beneficial use of microbes in association with AMB pathway. Biological air filtration (BAF) is a method currently being investigated for use during aircraft. This method reduces more than 99 % of toluene, chlorobenzene and dichloromethane in the air stream.

**Public health:**

AMP pathway is used for immunization against some disease like they are currently being used for influenza vaccines. However they are not widely used because they are painful.

**Bioaerosol control in laboratory:**

Bioaerosol containing airborne microbes can be controlled at every point by using different mechanism which includes:

**Ventilation:**

It is the most common method to check build up of airborne particles. This can be achieved by open windows or use of air conditioning and heating units that pump outside air into the room. This is cost effective and this will at least reduce the amount of microbes inside room.

**Filtration:**

Unidirectional air flow filtration is also simple and effective for bioaerosol control. HEPA is used for this purpose and it removes virtually all infectious particles. Bag house filtration has also become common in building

**Biocidal agents:**

These are used for super heating, super dehydration, ozonation and UV irradiation to eradicate the microorganisms. The most commonly used method is ultraviolet germicidal radiation (UVGI).

**Isolation:**

Is the enclosure of an environment through the use of positive or negative pressurized air gradients and air tight seals. Isolation chamber in TB wards in hospitals provide protection to other present inside the are air from these rooms is exhausted in to the atmosphere passing through a HEPA filter and biocidal control chamber.
This system work on negative pressurized air. Positive –pressure isolation chambers, working on the opposite principle force air out of the room thus protects occupants of the room from outside contamination.

**Factors affecting microbial survival in air:**

Many environmental factors have been shown to influence the ability of microorganism to survive the most important of them are given below:

**Atmospheric humidity:**

The relative as well as the absolute humidity content of the air play a major role in the survival of the air borne microorganism. In general it has been reported that most gram•negative bacteria associated with aerosols tend to survive for longr periods at relative low humidity by regulating their metabolic activities. This tends to be opposite for gram• positive bacteria. However at 100% relative humidity, longer exposure decreases the viability vis•à•vis survival. One mechanism that explains loss of viability in association with very low relative humidity is structural change in the lipid bilayers of the cell membrane. Intracellular ionic imbalance and loss of cellular metabolites occur when the cell is exposed to unfavorable humidity level. Viruses with enveloped nucleocapsids tend to have better survival in aerosols than without.

**Temperature:**

Temperature is the major factor in the inactivation of microbes. High temperature promotes inactivation, mainly associated with desiccation and protein denaturation and lower temperature promotes longer survival times. When temperatue approaches freezing, however, some organisms lose viability because of formation of ice crystals on their surfaces. The metabolic activities of microbes in air show a diurnal fluctuations in proportion to temperature fluctuations.

**Enumeration of Microorganisms in Air:**

There are several methods, which require special devices, designed for the enumeration of microorganisms in air. The most important ones are solid and liquid impingement devices, filtration, sedimentation, centrifugation, electrostatic precipitation, etc. However, none of these devices collects and counts all the microorganisms in the air sample tested. Some microbial cells are destroyed and some entirely pass through in all the processes. Some of the methods are described below.

**Impingement in liquids:** In this method, the air drawn is through a very small opening or a capillary tube and bubbled through the liquid. The organisms get trapped in the liquid medium. Aliquots of the liquid are then plated to determine its microbial content. Aliquots of the broth are then plated to determine microbial content.

**Impingement on solids:** In this method, the microorganisms are collected, or impinged directly on the solid surface of agar medium. Colonies develop on the medium where the organism impinges. Several devices are used, of which the settling•plate technique is the simplest, in this method the cover of the pertri dish containing an. agar medium is removed, and the agar surface is exposed to the air for several minutes. A certain number of colonies develop on incubation of the petridish.

Each colony represents particle carrying microorganisms. Since the technique does not record the volume of air actually sampled, it gives only a rough estimate. However, it does give information about the kind of microorganisms in a particular area. Techniques wherein a measured. Volume of air is sampled have also been developed. These are sieve and slit type devices. A sieve device has a large number of small holes in a metal cover, under which is located a Petri dish containing an agar medium A measured volume of air is drawn, through these small holes. Airborne particles impinge upon the agar surface. The plates are incubated and the colonies counted. In a slit device the air is drawn through a very narrow slit onto a Petri dish containing agar medium. The slit is approximately the length of the Petri dish. The Petri dish is rotated at a particular speed under the slit one complete turn is made during the sampling operation.

**Centrifugation:**

Air is sucked into a conical tube to create a vortex of sufficient velocity that particles are sedimented into a liquid trap at the base. In this figure air is drawn into the sampler at an angle (tangential) to the walls of the device so that it circulates around and down the wall. As it circulates decrease in the diameter of the sampling

body causes a dramatic increase in the velocity of the air and subsequently on the particle's terminal velocity. This increase in gravitational settling potential causes the particles to be trapped in the lower chamber because their 'centrifugally increased' mass prevents them from exiting with the return air flow.

**Air-sampling methods:**

Air sampling is used routinely to monitor the populations of airborne particles, and to inform the public about air quality and pollen/spore counts through public broadcasting (weather reports, etc.). It is used by major hospitals to monitor the populations of specific allergenic particles (fungal spores, etc.), so that the causes of patients' allergies can be determined. And it is used in crop pathology for disease•forecasting, so that growers can apply fungicides as and when required.

**The rotorod sampler** is a cheap, simple and portable air sampler. It consists of a U•shaped metal rod attached by a spindle to a battery•powered electric motor. The motor causes the upright arms of the metal rod to rotate at high speed. To use the sampler, the upright arms are covered with narrow strips of sticky tape, so that any spores in the air will impact onto the tapes. Then the tapes are removed and examined microscopically to identify the spores and other particles such as pollen grains in the air.One of the advantages of the rotorod sampler is that it can be used to precisely locate a source of spores of a particular fungus. The famous aerobiologist, PH Gregory, did this in the 1950s by placing rotorod samplers at different positions in a field and "homing in" on a source of spores of the fungus Pithomyces chartarum, which causes a condition known as facial eczema of sheep.

**The Burkard spore trap**

The Burkard spore sampler acts on the same principle as the rotorod sampler, but is used to give a continuous record of particles in the air over a period of 24 hours or up to 7 days. The apparatus (Figures J, K) consists of an air•sealed drum that contains a clockwork rotating disc (arrowhead in Fig. K) which makes a single revolution in 7 days. The surface of this disc is covered with adhesive tape, to trap spores that impact onto it. When the apparatus is assembled, air is sucked into the drum at high speed through a slit orifice (arrowhead in Fig J) by means of a motor at the base of the apparatus.

Any particles in the air impact onto the sticky tape near the slit orifice, giving a record of the particles in the atmosphere at a specific time of day. At the end of a 7•day run, the tape is removed, cut into sections representing hourly or daily periods, then examined microscopically.

In this way, it is possible to distinguish clearly between night•released and day•released spores or other particles, and also to relate the types of particle to different weather conditions (e.g. humid or dry periods) while the apparatus was running. The Burkard spore trap is commonly used for continuous monitoring of spore or pollen loads in the air. For example, these traps are commonly sited on hospital roofs, meteorological stations, and other public buildings, and provide public information through TV and radio broadcasts. The principle is exactly the same as in the rotorod sampler because the trapping of particles is based on impaction. The limitations also are the same: only the larger particles with sufficient mass will impact on the tapes at the air speeds generated by this type of sampler.

**The Anderson sampler**

The Anderson sampler is an ingenious device for selectively trapping different sizes of particles according to their size (momentum). This sampler consists of a stack of 8 metal sections that fit together with ring seals to form an air•tight cylinder. Each metal section has a perforated base, and the number of perforations is the same in each section, but the size of these perforations is progressively reduced from the top of the column to the bottom. To use this sampler, open agar plates are placed between each metal section, resting on three studs

When fully assembled (with an open agar plate between each unit) an electric motor sucks air from the bottom of the unit, causing spore•laden air to enter at the top and to pass down through the cylinder

One of the interesting features of the Anderson sampler is that it mimics the deposition of spores (or other airborne particles) in the human respiratory tract (Figure O). For example, relatively large fungal spores and pollen grains tend to be trapped on the mucus•covered hairs of our nostrils, where they can cause "hay fever" symptoms in sensitized individuals. Smaller particles are not trapped in the nostrils but instead are carried down into the bronchioles and alveoli. Here the air speed is very low, because the successive branching of the respiratory tract has reduced the air speed to a minimum. But spores of about 2•4 micrometers diameter can settle onto the mucosal surfaces of the alveoli. Some of these spores are important in initiating infections of the lungs.

However, it is important to note that the underlying mechanisms of spore deposition in the Anderson sampler are entirely different from those in the human respiratory tract • the Anderson sampler traps spores by impaction, whereas spores are deposited in human respiratory tract mainly by sedimentation.

**Significance of Microorganisms in Air:**

As long as microorganisms remain in the air they are of little importance. When they come to rest they may develop and become beneficial or harmful. Knowledge of the microorganisms in air is of importance in several aspects.

**Normal Microbiota of the Human Body**

In a healthy human the internal tissues (e.g., brain, blood, cerebrospinal fluid, muscles) are normally free of microorganisms. Conversely, the surface tissues (e.g., skin and mucous membranes) are constantly in contact with environmental microorganisms and become readily colonized by certain microbial species. The mixture of microorganisms regularly found at any anatomical site is referred to as the normal microbiota, the indigenous microbial population, the microflora, or the normal flora. For consistency, the term normal microbiota is used in this chapter. An overview of the microbiota native to different regions of the body and an introduction to the microorganisms one can expect to find on culture reports is presented next. Because bacteria make up most of the normal microbiota, they are empha• sized over the fungi (mainly yeasts) and protozoa.

There are many reasons to acquire knowledge of the normal human microbiota. Four specific examples include:

1. An understanding of the different microorganisms at specific locations provides greater insight into the possible infections that might result from injury to these body sites.

2. A knowledge of the normal microbiota in an infected part of the body gives the physician•investigator a better perspective concerning the possible source and significance of microorganisms isolated from an infection site.

3. A knowledge of the normal microbiota helps the physician• investigator understand the causes and consequences of colonization and growth by microorganisms normally absent at a specific body site.

4. An increased awareness of the role that these normal microbiota play in stimulating the host immune response can be gained. This awareness is important because the immune system provides protection against potential pathogens.

**Distribution of the Normal Microbiota**

Three of the most important types of sym• biotic relationships are commensalism, mutualism, and parasitism. Within each category the association may be either ectosymbiotic or endosymbiotic. In ectosymbiosis one organism remains outside the other. In endosymbiosis one organism is present within the other. In the following subsections, examples will be presented of both ecto and endosymbiotic relationships that exist with the human host. Both commensalistic and mutualistic relationships are considered.

**Skin**

The adult human is covered with approximately 2 square meters of skin. It has been estimated that this surface area supports about $10^{\bullet 12}$ bacteria. A commensal is an organism that participates in commensalism. Commensalism is a symbiotic relationship in which one species benefits and the other is unharmed. Commensal microorganisms living on or in the skin can be either resident (normal) or transient microbiota. Resident organisms normally grow on or in the skin. Their presence be• comes fixed in well•defined distribution patterns. Those microorganisms that are temporarily present are transients. Transients usually do not become firmly entrenched; they are unable to multiply and normally die in a short time.

The anatomy and physiology of the skin vary from one part of the body to another, and the normal resident microbiota reflect these variations. The skin surface or epidermis is not a favorable environment for microbial colonization. Several factors are responsible for this hostile microenvironment. First, the skin is subject to periodic drying. Lack of moisture drives many resident microbiota into a dormant state. However, in certain parts of the body (scalp, ears, axillary areas, genitourinary and anal regions, perineum, palms), moisture is sufficiently high to support a resident microbiota. Second, the skin has a slightly acidic pH due to the organic acids produced by normal staphylococci and secretions from skin oil and sweat glands. The acidic pH (4 to 6) discourages colonization by many microorganisms. Third, sweat contains a high concentration of sodium chloride. This makes the skin surface hyperosmotic and osmotically stresses most microorganisms. Finally, certain inhibitory substances (bactericidal and/or bacteriostatic) on the skin help control colonization, overgrowth, and infection from microorganisms. For example, the sweat glands re• lease lysozyme (muramidase), an enzyme that lyses Staphylococ• cus epidermidis and other gram•positive bacteria by hydrolyzing the þ (1□4) glycosidic bond connecting N-acetylmuramic acid and N-acetylglucosamine in the bacterial cell wall peptidoglycan The oil glands secrete complex lipids that may be partially degraded by the enzymes from certain gram•positive bacteria (Propionibacterium acnes). These bacteria can change the secreted
lipids to unsaturated fatty acids such as oleic acid that have strong antimicrobial activity against gram•negative

bacteria and some fungi. Some of these fatty acids are volatile and may be associated with a strong odor. Therefore many body deodorants contain antibacterial substances that act selectively against gram• positive bacteria to reduce the production of volatile unsaturated fatty acids and body odor. However, deodorants can shift the normal microbiota to predominantly gram•negative bacteria and precipitate subsequent infections.

Most skin bacteria are found on the superficial cells, colonizing dead cells, or closely associated with the oil and sweat glands. Secretions from these glands provide the water, amino acids, urea, electrolytes, and specific fatty acids that serve as nutrients primarily for Staphylococcus epidermidis and aerobic corynebacteria. Gram• negative bacteria generally are found in the moister regions. The yeasts Pityrosporum ovale and P. orbiculare normally occur on the scalp. Some dermatophytic fungi may colonize the skin and produce athlete's foot and ringworm. Athlete's foot disease.

The most prevalent bacterium in the skin glands is the gram• positive, anaerobic, lipophilic rod Propionibacterium acnes. This bacterium usually is harmless; however, it has been associated with the skin disease acne vulgaris. Acne commonly occurs during adolescence when the endocrine system is very active. Hormonal activity stimulates an overproduction of sebum, a fluid secreted by the oil glands. A large volume of sebum accumulates within the glands and provides an ideal microenvironment for P. acnes. In some individuals this accumulation triggers an inflammatory re- sponse that causes redness and swelling of the gland's duct and produces a comedo [pl., comedones], a plug of sebum and keratin in the duct. Inflammatory lesions (papules,pustules, nodules) commonly called "blackheads" or "pimples" can result. P. acnes produces lipases that hydrolyse the sebum triglycerides into free fatty acids. Free fatty acids are especially irritating because they can en• ter the dermis and promote inflammation. Because P. acnes is ex• tremely sensitive to tetracycline, this antibiotic may aid acne sufferers. Accutane, a synthetic form of vitamin A, is also used.

Some pathogens found on or in the skin are residents that colonize the area around orifices. Staphylococcus aureus is the best example. It resides in the nostrils and perianal region but survives poorly elsewhere. In like manner Clostridium perfringens usually colonizes only the perineum and thighs, especially in those who suffer from diabetes.

**Nose and Nasopharynx**

The normal microbiota of the nose is found just inside the nostrils. Staphylococcus aureus and S. epidermidis are the predomi• nant bacteria present and are found in approximately the same numbers as on the skin of the face.

The nasopharynx, that part of the pharynx lying above the level of the soft palate, may contain small numbers of potentially pathogenic bacteria such as Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenzae. Diphtheroids, a large group of nonpathogenic gram•positive bacteria that resemble Corynebacterium, are commonly found in both the nose and nasopharynx.

**Oropharynx**

The oropharynx is that division of the pharynx lying between the soft palate and the upper edge of the epiglottis. Like the nose, large numbers of Staphylococcus aureus and S. epidermidis in• habit this region. The most important bacteria found in the oropharynx are the various alpha•hemolytic streptococci (S. oralis, S. milleri, S. gordonii, S. salivarius); large numbers of diphtheroids; Branhamella catarrhalis; and small gram• negative cocci related to Neisseria meningitidis. It should be noted that the palatine and pharyngeal tonsils harbor a similar microbiota, except that within the tonsillar crypts, there is an increase in Micrococcus and the anaerobes Porphyromonas, Prevotella, and Fu sobacterium. (Porphyromonas spp. and Prevotella spp. were formerly classified as Bacteroides.)

**Respiratory Tract**

The upper and lower respiratory tracts (trachea, bronchi, bron• chioles, alveoli) do not have a normal microbiota. This is because microorganisms are removed by (1) the continuous stream of mu• cus generated by the ciliated epithelial cells and (2) the phagocytic action of the alveolar macrophages. In addition, a bacterici• dal effect is exerted by the enzyme lysozyme, present in nasal mucus. Mucociliary blanket

**Mouth**

The normal microbiota of the mouth or oral cavity contains organisms able to resist mechanical removal by adhering to surfaces like the gums and teeth. Those that cannot attach are removed by the mechanical flushing of the oral cavity contents to the stomach where they are destroyed by hydrochloric acid. The continuous desquamation (shedding) of epithelial cells also removes mi• croorganisms. Those microorganisms able to colonize the mouth find a very comfortable environment due to the availability of wa• ter and nutrients, the suitability of pH and temperature, and the presence of many other growth factors. The oral cavity is colonized by microorganisms from the sur• rounding environment within hours after a human is born. Initially the microbiota consists mostly of the genera Streptococcus, Neis• seria, Actinomyces, Veillonella, and Lactobacillus. Some yeasts also are present. Most microorganisms that invade the oral cavity initially are aerobes and obligate anaerobes. When the first teeth erupt, the anaerobes (Porphyromonas, Prevotella, and Fusobac• terium) become dominant due to the anaerobic nature of the space between the teeth and gums. As the teeth grow, Streptococcus parasanguis and S. mutans attach to their enamel surfaces; S. salivarius attaches to the buccal and gingival epithelial surfaces and colonizes the saliva. These streptococci produce a glycocalyx and various other adherence factors that enable them to attach to oral surfaces. The presence of these bacteria contributes to the eventual formation of dental plaque, caries, gingivitis, and periodontal disease.

**Eye**

At birth and throughout human life, a small number of bacterial com• mensals are found on the conjunctiva of the eye. The predominant bacterium is Staphylococcus epidermidis followed by S. aureus, aerobic corynebacteria (diphtheroids), and Streptococcus pneumoniae. Cultures from the eyelids or conjunctiva also yield Branhamella ca• tarrhalis, Escherichia, Klebsiella, Proteus, Enterobacter, Neisseria, and Bacillus species. Few anaerobic organisms are present.

**External Ear**

The norma microbiota of the external ear resemble those of the skin, with coagulase•negative staphylococci and Corynebacterium predominating. Less frequently found are Bacillus, Micrococcus, and Neisseria species. Gram•negative rods such as Proteus, Escherichia, and Pseudomonas are occasionally seen. Mycological studies show the following fungi to be normal microbiota: Aspergillus, Alternaria, Penicillium, Candida, and Saccharomyces.

**Stomach**

As noted earlier, many microorganisms are washed from the mouth into the stomach. Owing to the very acidic pH values (2 to 3) of the gastric contents, most microorganisms are killed. As a result the stomach usually

contains less than 10 viable bacteria per milliliter of gastric fluid. These are mainly Sarcina, Streptococcus, Staphylococcus, Lactobacillus, Peptostreptococcus, and yeasts such as Candida spp. Microorganisms may survive if they pass rapidly through the stomach or if the organisms ingested with food are par• ticularly resistant to gastric pH (mycobacteria). Normally the num• ber of microorganisms increases after a meal but quickly falls as the acidic pH takes its toll. Changes in the gastric microbiota also oc• cur if there is an increase in gastric pH following intestinal obstruction, which permits a reflux of alkaline duodenal secretions into the stomach. If the gastric pH increases, the microbiota of the stomach are likely to reflect that of the oropharynx and, in addition, contain both gram•negative aerobic and anaerobic bacteria.

**Small Intestine**

The small intestine is divided into three anatomical areas: the duodenum, jejunum, and ileum. The duodenum (the first 25 cm of the small intestine) contains few microorganisms because of the combined influence of the stomach's acidic juices and the inhibitory action of bile and pancreatic secretions. Of the bacteria present, gram•positive cocci and rods comprise most of the mi• crobiota. Enterococcus faecalis, lactobacilli, diphtheroids, and the yeast Candida albicans are occasionally found in the je crobiota begin to take on the characteristics of the colon micro• biota. It is within the ileum that the pH becomes more alkaline. As a result anaerobic gram•negative bacteria and members of the family Enterobacteriaceae become established.

**Large Intestine (Colon)**

The large intestine or colon has the largest microbial community in the body. Microscopic counts of feces approach $10^{\bullet 12}$ organisms per gram wet weight. Over 400 different species have been iso• lated from human feces. The colon can be viewed as a large fer• mentation vessel, and the microbiota consist primarily of anaerobic, gram•negative, nonsporing bacteria and gram•positive, spore•forming, and nonsporing rods. Not only are the vast major• ity of microorganisms anaerobic, but many different species are present in large numbers. Several studies have shown that the ratio of anaerobic to facultative anaerobic bacteria is approximately 300 to 1. Even the most abundant of the latter, Escherichia coli, is only about 0.1% of the total population.

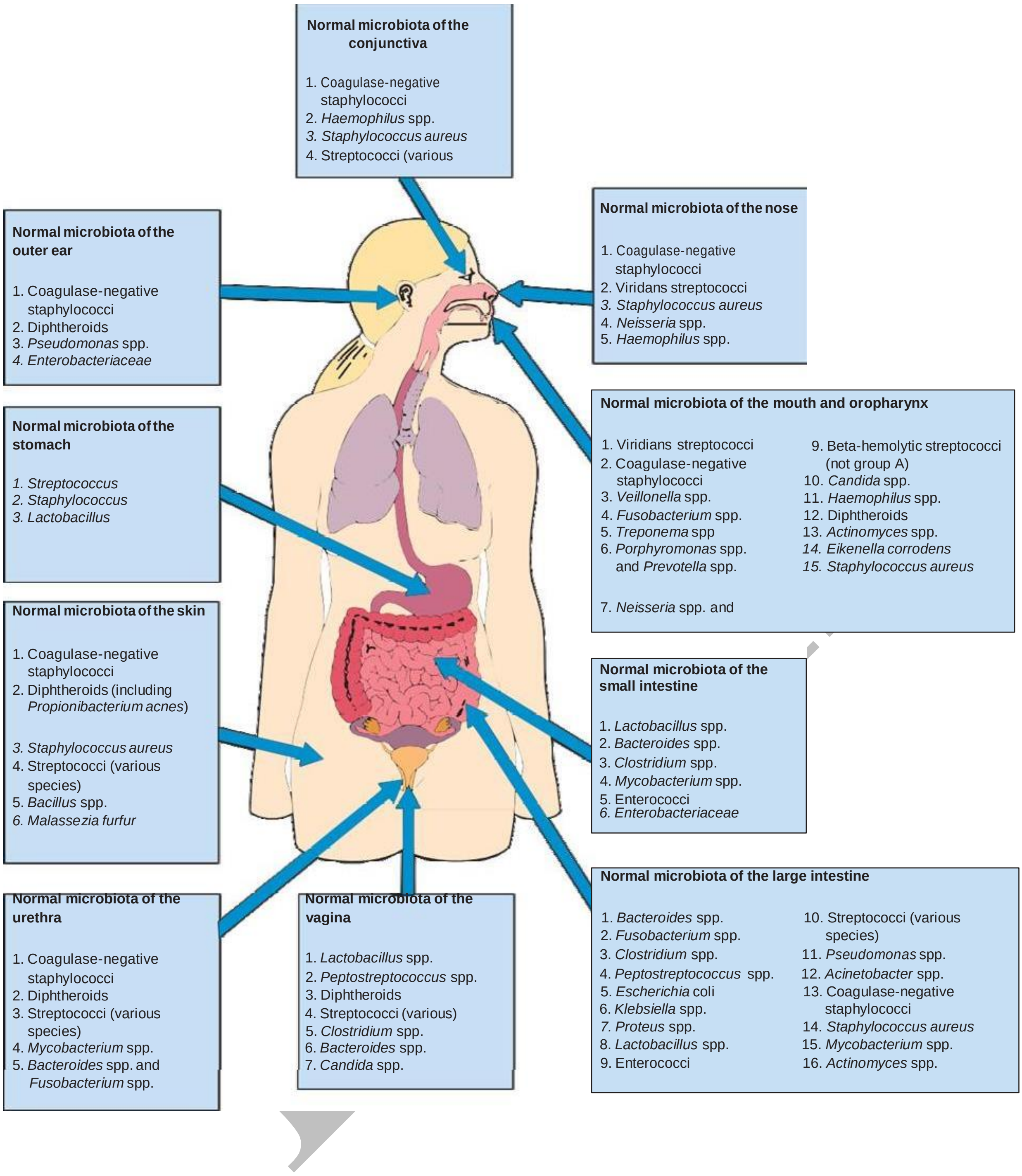
Besides the many bacteria in the large intestine, the yeast Candida albicans and certain protozoa may occur as harmless commensals. Trichomonas hominis, Entamoeba hartmanni, Endolimax nana, and Iodamoeba butschlii are common inhabitants.Various physiological processes move the microbiota through the colon so an adult eliminates about $3 \times 10^{\bullet 13}$ microorganisms daily. These processes include peristalsis and desquamation of the surface epithelial cells to which microorganisms are attached, and continuous flow of mucus that carries adhering microorganisms with it. To maintain homeostasis of the microbiota, the body must continually replace those lost microorganisms. The bacterial popu• lation in the human colon usually doubles once or twice a day. Under normal conditions the resident microbial community is self• regulating. Competition and mutualism between different microorganisms and between the microorganisms and their host serve to maintain a status quo. However, if the intestinal environment is disturbed, the normal microbiota may change greatly. Disruptive factors include stress, altitude changes, starvation, parasitic organisms, diarrhea, and use of antibiotics or probiotics. Finally, it should be emphasized that the actual proportions of the individual bacterial populations within the indigenous microbiota depend largely on a person's diet.

The initial residents of the colon of breast•fed infants are members of the gram•positive Bifidobacterium genus, because human milk contains a disaccharide amino sugar that Bifidobacterium species require as a growth factor. In formula•fed infants, gram•positive Lactobacillus species predominate because formula lacks the required growth factor. With the ingestion of solid food, these initial colonizers of the colon are eventually displaced by a typical gram-negative microbiota. Ultimately the composition of the adult's microbiota is established.

**Genitourinary Tract**

The upper genitourinary tract (kidneys, ureters, and urinary bladder) is usually free of microorganisms. In both the male and fe• male, a few bacteria (Staphylococcus epidermidis, Enterococcus faecalis, and Corynebacterium spp.) usually are present in the distal portion of the urethra. Neisseria and some members of the Enterobacteriaceae are occasionally found.

In contrast, the adult female genital tract, because of its large surface area and mucous secretions, has a complex microbiota that constantly changes with the female's menstrual cycle. The major microorganisms are the acid•tolerant lactobacilli, primarily Lactobacillus acidophilus, often called Döderlein's bacilli. They ferment the glycogen produced by the vaginal epithelium, form• ing lactic acid. As a result the pH of the vagina and cervix is main• tained between 4.4 and 4.6.


Normal microbiota of the conjunctiva
1. Coagulase-negative staphylococci
2. Haemophilus spp.
3. Staphylococcus aureus
4. Streptococci (various
Normal microbiota of the outer ear
1. Coagulase-negative staphylococci
2. Diphtheroids
3. Pseudomonas spp.
4. Enterobacteriaceae
Normal microbiota of the nose
1. Coagulase-negative staphylococci
2. Viridans streptococci
3. Staphylococcus aureus
4. Neisseria spp.
5. Haemophilus spp.
Normal microbiota of the mouth and oropharynx
1. Viridians streptococci
2. Coagulase-negative staphylococci
3. Veillonella spp.
4. Fusobacterium spp.
5. Treponema spp
6. Porphyromonas spp. and Prevotella spp.
7. Neisseria spp. and
9. Beta-hemolytic streptococci (not group A)
10. Candida spp.
11. Haemophilus spp.
12. Diphtheroids
13. Actinomyces spp.
14. Eikenella corrodens
15. Staphylococcus aureus
Normal microbiota of the stomach
1. Streptococcus
2. Staphylococcus
3. Lactobacillus
Normal microbiota of the skin
1. Coagulase-negative staphylococci
2. Diphtheroids (including Propionibacterium acnes)
3. Staphylococcus aureus
4. Streptococci (various species)
5. Bacillus spp.
6. Malassezia furfur
Normal microbiota of the small intestine
1. Lactobacillus spp.
2. Bacteroides spp.
3. Clostridium spp.
4. Mycobacterium spp.
5. Enterococci
6. Enterobacteriaceae
Normal microbiota of the urethra
1. Coagulase-negative staphylococci
2. Diphtheroids
3. Streptococci (various species)
4. Mycobacterium spp.
5. Bacteroides spp. and Fusobacterium spp.
Normal microbiota of the vagina
1. Lactobacillus spp.
2. Peptostreptococcus spp.
3. Diphtheroids
4. Streptococci (various)
5. Clostridium spp.
6. Bacteroides spp.
7. Candida spp.
Normal microbiota of the large intestine
1. Bacteroides spp.
2. Fusobacterium spp.
3. Clostridium spp.
4. Peptostreptococcus spp.
5. Escherichia coli
6. Klebsiella spp.
7. Proteus spp.
8. Lactobacillus spp.
9. Enterococci
10. Streptococci (various species)
11. Pseudomonas spp.
12. Acinetobacter spp.
13. Coagulase-negative staphylococci
14. Staphylococcus aureus
15. Mycobacterium spp.
16. Actinomyces spp.

An **extremophile** (from Latin extremus meaning "extreme" and Greek philiā (φιλία) meaning "love") is an organism that thrives in physically or geochemically extreme conditions that are detrimental to most life on Earth. In contrast, organisms that live in more moderate environments may be termed mesophiles or neutrophiles. There are many classes of extremophiles that range all around the globe, each corresponding to the way its environmental niche differs from mesophilic conditions. These classifications are not exclusive. Many extremophiles fall under multiple categories and are classified as polyextremophiles. For example, organisms living inside hot rocks deep under Earth's surface are thermophilic and barophilic such as *Thermococcus barophilus*. A polyextremophile living at the summit of a mountain in the Atacama Desert might be a radioresistant xerophile, a psychrophile, and an oligotroph. Polyextremophiles are well known for their ability to tolerate both high and low pH levels. Extremophiles live in conditions outside the moderate environments most life requires. From high•temperature, deep sea vents to freezing, polar oceans, we'll explore life at some of the most extreme environments on Earth.

From tiny, single•celled organisms to complex creatures such as ourselves, most of the wondrous life on Earth thrives in moderate conditions. These are environments where it is 'not too hot, not too cold, but just right,' to take a line from the fairytale Goldilocks. Moderate conditions can also refer to salt content, or pH (a measure of how acidic or how basic an environment is). Most organisms require an environment that has a more neutral pH, 'not too acidic, not too basic, but just right.' However, a whole class of organisms not only survives in extreme conditions, they thrive! Extremophiles (which loosely translates to 'lovers of extremes') are adapted to what is considered on Earth to be an extreme environment. From Antarctic ice to hydrothermal vents, extremophiles can be adapted to live in extreme cold, intense heat, harsh acidity, high saltiness and a host of other conditions in which we humans have been surprised to detect life at all.

**Types of Extremophiles**

Extremophiles are adapted to their particular extreme environment; it's not just that they can live there. To thrive, extremophiles must live in their special environment. For example, if a human put on a thick parka and spent some time in Antarctica, it wouldn't make a human an extremophile. An extremophile is genetically adapted to its extreme environment. That means it's unlikely to survive in moderate conditions.

**Thermophiles**

are heat•loving, and are found in environments like deep sea vents, volcanic soil, and around geysers.

**Psychrophiles,**

also known as cryophiles, are just the opposite. These organisms are adapted to cold, and live in places like polar seas.

**Halophiles**

thrive in high salt conditions. They live in brine, and may be found in salt flats or lakes. An organism requiring at least 0.2M concentrations of salt (NaCl) for growth

**Acidophiles**

are adapted to extremely acidic conditions, such as volcanic landscapes. An organism with optimal growth at pH levels of 3 or below

If an organism is adapted to multiple extreme conditions, it's labeled a polyextremophile. For example, a thermoacidophile is adapted to hot (thermo), acidic (acido) conditions.

**Alkaliphile**

An organism with optimal growth at pH levels of 9 or above

**Anaerobe**

An organism that does not require oxygen for growth such as *Spinoloricus cinzia*. Two sub•types exist: facultative anaerobe and obligate anaerobe. A facultative anaerobe can tolerate anaerobic and aerobic conditions; however, an obligate anaerobe would die in the presence of even trace levels of oxygen

**Cryptoendolith**

An organism that lives in microscopic spaces within rocks, such as pores between aggregate grains; these may also be called Endolith, a term that also includes organisms populating fissures, aquifers, and faults filled with groundwater in the deep subsurface

**Hyperthermophile**

An organism that can thrive at temperatures above 80 °C, such as those found in hydrothermal systems

**Hypolith**

An organism that lives underneath rocks in cold deserts

**Lithoautotroph**

An organism (usually bacteria) whose sole source of carbon is carbon dioxide and exergonic inorganic oxidation (chemolithotrophs) such as *Nitrosomonas europaea*; these organisms are capable of deriving energy from reduced mineral compounds like pyrites, and are active in geochemical cycling and the weathering of parent bedrock to form soil

**Metallotolerant**

Capable of tolerating high levels of dissolved heavy metals in solution, such as copper, cadmium, arsenic, and zinc; examples include *Ferroplasma sp.*, *Cupriavidus metallidurans* and GFAJ•1

**Oligotroph**

An organism capable of growth in nutritionally limited environments

**Osmophile**

An organism capable of growth in environments with a high sugar concentration

**Piezophile**

(Also referred to as barophile). An organism that lives optimally at high pressures such as those deep in the ocean or underground, common in the deep terrestrial subsurface, as well as in oceanic trenches

**Polyextremophile**

A polyextremophile (faux Ancient Latin/Greek for 'affection for many extremes') is an organism that qualifies as an extremophile under more than one category

**Psychrophile/Cryophile**

An organism capable of survival, growth or reproduction at temperatures of •15 °C or lower for extended periods; common in cold soils, permafrost, polar ice, cold ocean water, and in or under alpine snowpack

**Radioresistant**

Organisms resistant to high levels of ionizing radiation, most commonly ultraviolet radiation, but also including organisms capable of resisting nuclear radiation

**Thermophile**

An organism that can thrive at temperatures between 45–122 °C

**Thermoacidophile**

Combination of thermophile and acidophile that prefer temperatures of 70–80 °C and pH between 2 and 3

**Xerophile**

An organism that can grow in extremely dry, desiccating conditions; this type is exemplified by the soil microbes of the Atacama Desert

**icrobial sucession in decomposition of organic matter**

Microbiology of decomposition is the study of all microorganisms (mainly bacteria and fungi) involved in decomposition, the chemical and physical processes during which organic matter is broken down and reduced to its original elements.

Decomposition microbiology can be divided between two fields of interest:

Decomposition of plant materials;

Decomposition of cadavers and carcasses.

The decomposition of plant materials is commonly studied in order to understand the cycling of carbon within a given environment and to understand the subsequent impacts on soil quality. Plant material decomposition is also often referred to as composting.

**Decomposition microbiology of plant materials**

The breakdown of vegetation is highly dependent on oxygen and moisture levels. During decomposition, microorganisms require oxygen for their respiration. If anaerobic conditions dominate the decomposition environment, microbial activity will be slow and thus decomposition will be slow. Appropriate moisture levels are required for microorganisms to proliferate and to actively decompose organic matter. In arid environments, bacteria and fungi dry out and are unable to take part in decomposition. In wet environments, anaerobic conditions will develop and decomposition can also be considerably slowed down. Decomposing microorganisms also require the appropriate plant substrates in order to achieve good levels of decomposition. This usually translates to having appropriate carbon to nitrogen ratios (C: N). The ideal composting carbon•to• nitrogen ratio is thought to be approximately 30:1. As in any microbial process, the decomposition of plant litter by microorganisms will also be dependent on temperature. For example, leaves on the ground will not undergo decomposition during the winter months where snow cover occurs as temperatures are too low to sustain microbial activities.

**ENVIRONMENTAL MICROBIOLOGY - 18MBU403**

**Possible Questions – Unit I**

1. Write a note on ecosystem.
2. Discuss in detail about air-borne fungal diseases.
3. Write a note on fresh water ecosystem.
4. Give an account on marine water ecosystem.
5. Comment on types of solid and liquid wastes characterization.
6. Briefly write a note on decomposition of plant organic matter.
7. Sketch a note on utilization of solid wastes (SCP and mushroom).
8. Write short notes on subterranean microbes and bioremediation.
9. Detail notes on positive and negative roles of microbes in environment
10. Comment on facors affecting microbial growth.

# KARPAGAM ACADEMY OF HIGHER EDUCATION



| Sno | Question | Option 1 | Option 2 | Option 3 | Option 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | The portion of the earth and its environment which can support life is known as __________ | Crust | Biosphere | Exosphere | atmosphere | Biosphere |
| 2 | The main energy source for the environment is _________ | Solar energy | Chemical energy | Bioelectric energy | Electrical energy | Solar energy |
| 3 | Effective air sanitizing is done by | Gamma radiation | UV radiation | Beta radiation dma radiation | Nano radiation | UV radiation |
| 4 | The principle involved in the air sampler Hesses tube is | Centrifugal action | Settling under gravity | Filltration | impingement | Settling under gravity |
| 5 | The principle involved in Litton air sampler is | Centrifugal action | Electrostatic precipitation | Filtration | impingement | Centrifugal action |
| 6 | Who first showed that microorganisms could occur as airborne contaminants | Koch | Spallanzani | Schwann | Pasteur | Pasteur |
| 7 | Droplet nuclei are significant in the transmission of diseases of the | Digestive system | Nervous system | Reproductive system | Respiratory system | Respiratory system |
| 8 | Air borne infections are transmitted mainly by | Aerobes from person to person | Inhaling spores or hyphal fragments from soil or dead vegetation | Drinking contaminated water | Objects such as handkerchiefs that are contaminated with respiratory secretations | Aerobes from person to person |
| 9 | The atmospheric layer nearest to the earth is | Troposphere | Stratosphere | Lithosphere | None of the above | Troposphere |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10 | Air mainly acts a_____________ medium for microorganisms | transport or dispersal | Growth medium | sterilised medium | none of the above | transport or dispersal |
| 11 | Usually _________________ are present at very high altitudes. | Spores | Vegetative cells | Mycelium | viruses | Mycelium |
| 12 | The air found inside the building is reffered to as | Indoor air | out door air | sterlised air | contaminated air | Indoor air |
| 13 | The common problem associated with Nosocomial pathogens is their | Antibiotic resistance | Dysentry | Diarrhoea | Fever | Antibiotic resistance |
| 14 | The filtering material used in granular filter is | Activated charcoal | Glass wool | ore | calcium carbonate | Activated charcoal |
| 15 | Of the different atmospheric layers ___________is characterized by a heavy load of microorganisms | Troposphere | Stratosphere | Lithosphere | None of the above | Troposphere |
| 16 | The type of isolation in which susceptible persons are separated from the source is | Protective isolation | cubic isolation | source isolation | none of the above | Protective isolation |
| 17 | ________________may act as more potential agents of infectious diseases than droplets | Droplet nuclei | Dust particles | Aerosols | none of the above | Droplet nuclei |
| 18 | ___________________are relatively more abundant than the vegetative cells in the air | Spores | Infectious dust | Aerosols | Droplets | Spores |
| 19 | ______________is an occupational disease | Brucellosis | Pulmanory disease | Pneumonitis | Meningitis | Brucellosis |
| 20 | ____________removes almost all microorganisms from atmosphere | Rainfall | pH | Chemicals | Mutagenesis | Rainfall |

| | | | | | | |
|---|---|---|---|---|---|---|
| 21 | Spores of ___________________travel over a thousand kilometers | Clostridium perfringens | Puccinia graminis | Sarcina lutea | Micrococcus luteus | Clostridium perfringens |
| 22 | Farmer's lung caused by exposure to spores of thermophilic _________ | Fungi | Bacteria | Actinomycetes | Viruses | Actinomycetes |
| 23 | HEPA filters are typically rated as ________ effective in removing dust. | 90 | 99.92% | 99.97% | 90.99% | 99.97% |
| 24 | In Lemon sampler air is drawn at the rate of_______________per minute and dispersed through the broth | 20-25litre | 25-30litre | 30-35litre | 35-40litre | 20-25litre |
| 25 | Laminar airflow developed by ___________. | Whittaker | Whitifield | Tyndall | Koch | Whitifield |
| 26 | Lilton large volume air sampler is an example for ____________. | Sieve sampler | Impingement | Electrostatic precipitation | Centrifugal action | Electrostatic precipitation |
| 27 | Of the different atmospheric layers ________is characterized by a heavy load of microorganisms | Troposphere | Stratosphere | Lithosphere | Atmosphere | Troposphere |
| 28 | Slit sampler can collect upto ______% of the water droplet particles sprayed into air | 85% | 100% | 95% | 75% | 85% |
| 29 | The amount of carbondioxide present in the atmosphere is near to | 0.02% | 0.03% | 0.04% | 0.05% | 0.04% |
| 30 | The dominant genera of common saprophytic fungi in indoor air is | Aspergillus | Fusarium | Penicillium | Mucor | Penicillium |

| | | | | | | |
|---|---|---|---|---|---|---|
| 31 | Viruses survive in the atmosphere at low temperature from | 8 to 32°C | 7 to 24°C | 6 to 18°C | 2 to 6°C | 6 to 18°C |
| 32 | ________ is a seasonal disease that can be deadly when becomes pandemic | Tuberculosis | Leprosy | Food poisoning | Influenza | Influenza |
| 33 | _________ air sampler is used in electrostatic precipitation of removing particles from air | Impinger | Orange | Sutton small volume | Litton large volume | Litton large volume |
| 34 | _________ is a severe respiratory disease caused by bacteria | Aspergillosis | Scarlet fever | Tuberculosis | Tetanus | Tuberculosis |
| 35 | __________ is an opportunistic fungal disease of human caused by inhalation of spores | Sporidiasis | Penicilliosis | Aspergillosis | Candidiasis | Aspergillosis |
| 36 | _______________ is transmitted to humans by inhalation of faecal dust from pigeon droppings | Candidiasis | Cryptococcosis | Sporidiasis | Bacteraemia | Cryptococcosis |
| 37 | According to ambient air quality standards in India, amount of suspended particulate matters in a residential area is | 200 $\mu gm^{-3}$ | 400 $\mu gm^{-3}$ | 600 $\mu gm^{-3}$ | 800 $\mu gm^{-3}$ | 200 $\mu gm^{-3}$ |
| 38 | Air doesn't have ___________ flora | Indigenous | Endogenous | Exogenous | Subgenous | Indigenous |
| 39 | Aspergillosis | Human | Soil | Industries | Vehicles | Both I & II |
| 40 | Cryptococcosis is caused by | *C.neoformans* | *C.licheniformis* | *C.pseudopodis* | *C.parvum* | *C.neoformans* |

| | | | | | | |
|---|---|---|---|---|---|---|
| 41 | Efficiency of HEPA filter | 75% | 90% | 95% | 99.97% | 99.97% |
| 42 | HEPA is an | Water filter | Soil filter | Air filter | Smoke arrester | Air filter |
| 43 | HEPA stands for | High Efficiency Particulate Air | High Energy Particulate Air | High Emission Particulate Air | High Efficient Polar Aerosol | High Efficiency Particulate Air |
| 44 | Optimum rate of relative humidity for the survival of most microorganisms is between | 10-20% | 30-60% | 40-80 % | 80-100% | 40-80 % |
| 45 | Percentage of oxygen is atmospheric air | 40.9 | 60.9 | 75.9 | 20.9 | 20.9 |
| 46 | Residue of solid material left after drying up of a droplet is known as | Mucus | Karyon | Oocyst | Droplet nuclei | Droplet nuclei |
| 47 | Size of a droplet nuclei | 0.1-0.4 µm | 1-4 µm | 4-10 µm | 10-40 µm | 1-4 µm |
| 48 | Ozone is formed in the upper atmosphere by a photochemical reaction with _______ | Ultraviolet solar radiation | Infra red radiation | Visible light | Gamma rays | Ultra violet solar radiation |
| 49 | Identify the term that describes an environment completely free of microorganisms | Antibiotic | Asepsis | Antisepsis | Probiotic | Asepsis |
| 50 | Average salinity of seawater | 15 ppt | 25 ppt | 35 ppt | 45 ppt | 35 ppt |
| 51 | An example for common air borne epidemic disease | Influenza | Typhoid | Encephalitis | Malaria | Influenza |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 52 | Ecological region at the lowest level of a body of water such as an ocean or a lake | Limnetic zone | Littoral zone | Profoundal zone | Benthic zone | Benthic zone | |
| 53 | In a lake the combined littoral and limnetic zone is known as | Profundal zone | Euphotic zone | Metalimnon | Epilimnon | Epilimnon | |
| 54 | Main photosynthetic body of the lake | Littoral zone | Limnetic zone | Profoundal zone | Paleic zone | Limnetic zone | |
| 55 | Microorganisms found attached to the rock surface are reffered to as | Epipeplon | Episammon | Epixylon | Epilithon | Epipeplon | |
| 56 | Profoundal zone is | Open surface of water body | Sub-surface zone | Deepest zone | Side zone | Deepest zone | |
| 57 | Which of the following can be seen in marine environment? | Halophiles | Barophiles | Psychrophiles | Hydrophiles | Halophiles | |
| 58 | Which of the following is not common in marine environment? | Luminous bacteria | Psychrophilic bacteria | Thermophilic bacteria | Barophilic bacteria | Luminous bacteria | |
| 59 | Zone near shore area where sunlight penetrates the sediment and allows aquatic plants to grow | Littoral zone | Limnetic zone | Paleic zone | Profoundal zone | Littoral zone | |
| 60 | Mycobacterium tuberculosis was first discovered by | Robert Koch | Edward Jenner | Louis Pasteur | None of these | Robert Koch | |

**Interactions among soil microorganisms**

The microbial ecosystem is the sum of the biotic and abiotic components of soil. It includes the total microbial flora together with the physical composition and physical characteristics of the soil. The microorganisms that inhabit the soil exhibit many different types of interactions or associations. Some interactions are indifferent or neutral; while some are positive and some are negative in nature. The associations existing between different soil microorganisms, whether of a symbiotic or antagonistic nature, influence the activities of microorganisms in the soil.

**Neutral associations**

Neutral association or neutralism is the association between microorganisms, where two different species of microorganisms occupy the same environment without affecting each other. Such an association might be transitory; as conditions change in the environment, like nutrients availability, there might be a change in the relationship.

**Positive associations**

There are three types of positive associations exist between microorganisms, which are given below.

**Mutualism**

Mutualism is an example of a symbiotic relationship in which each organism benefits from the association. The way in which benefit is derived depends on the type of interactions. Syntrophism is a type of mutualistic association, which involves the exchange of nutrition between two species. The association between blue green algae and a fungus (lichen) is known as syntrophism.

The fungus surrounds the algal cells, often enclosing them within complex fungal tissues unique to lichen associations. In this type of association, algae benefits by protection afforded to it by the fungal hyphae from environmental stresses, while the fungus obtain and use CO2 released by the algae during photosynthesis. The algal or cyanobacterial cells are photosynthetic, and as in plants they reduce atmospheric carbon dioxide into organic carbon sugars to feed both symbionts. Both partners gain water and mineral nutrients mainly from the atmosphere, through rain and dust. The lichen association is a close symbiosis. It extends the ecological range of both partners but is not always obligatory for their growth and reproduction in natural environments, since many of the algal symbionts can live independently.

Another mutalistic association is characterized by different metabolic products from the association as compared with the sum of the products of the separate species. A mutualism between Thiobacillus ferrooxidans and Beijerinckia lacticogenes helps in ore leaching. Both of these species when grown in a medium free of added carbon and nitrogen sources and with a sterile ore concentrate, the growth of the two species in association and the resulting effect on the rate of leaching copper from the ore concentrate is observed.

Leaching is process of recovery of metal from the ore, where microorganisms play the important role of oxidizing insoluble metal sulphides to soluble sulphates.

Microorganisms may also form mutualistic relationships with plants in soil, an example of which is nitrogen fixing bacteria i.e. Rhizobium growing in the roots of legumes (Plants of the family leguminoceae). In this Rhizobium-legume association, Rhizobium bacteria are benefited by protection from the environmental stresses while in turn plant is benefited by getting readily available nitrate nitrogen released by the bacterial partner.

**Commensalism**

Commensalism refers to a relationship between organisms, in which one species of a pair benefits whereas the other is not affected. This happens commonly in soil with respect to degradation of complex molecules like cellulose and lignin. For example, many fungi can degrade cellulose to glucose, which is utilized by many bacteria. Many bacteria are unable to utilize cellulose, but they can utilise the fungal breakdown products of cellulose, e.g., glucose and organic acids.

Another example of commensalism is that of a change in the substrate produced by a combination of species and not by individual species. For example, lignin which is major constituent of woody plants and is usually resistant to degradation by most of the microorganisms. But in forest soils, lignin is readily degraded by a group of Basidiomycetous fungi and the degraded products are used by several other fungi and bacteria which can not utilize lignin directly.

**Proto-cooperation**

It is a mutually beneficial association between two species. Protocooperation is a form of mutualism, but they do not depend on each other for survival. An example of protocooperation happens between soil bacteria or fungi, and the plants that occur growing in the soil. None of the species rely on the relationship for survival, but all of the fungi, bacteria and higher plants take part in shaping soil composition and fertility. Soil bacteria and fungi interrelate with each other, forming nutrients essential to the plants survival. Plants utilize these microorganism synthesized nutrients through root nodules thereby decomposing organic substances. Soil bacteria and fungi help in improving the fertility of the soil and shaping of soil. Plants get essential carbon dioxide and nutrients. Nutritional proto-cooperation between bacteria and fungi has been reported for various vitamins, amino and purines in terrestrial ecosystem and are very useful in agriculture.

**Negative associations**

**Antagonism**

It is the relationship in which one species of an organism is inhibited or adversely affected by another species in the same environment. The relationship is also known as antagonism. The species which adversely affects the other is said to be antagonistic. Such organisms may be of great practical importance, since they often produce antibiotics or other inhibitory substances which affect the normal growth processes or survival of other organisms.

Antagonistic relations are most common in nature. One example of which is the antagonistic nature of both Staphylococcus aureus andPseudomonas aeruginosa towards the fungus Aspergillus terreus. Certain Pseudomonas pigments inhibit germination of Aspergillus spores.Staphylococcus aureus produces a diffusable antifungal material that causes distortions and hyphal swellings in Aspergillus terreus.

An antibiotic is a microbial inhibitor of biological origin. Soil microorganisms are the most common producers of antibiotics. Production of antibiotics in soil may enable the antibiotic producing organism to thrive successfully in a competitive environment. One example of which is the presence of large populations of actinomycetes in the chitinaceous shells of dead crustaceans in the sea. Their existence, in the environment free of other microorganisms, is may be due to the production of antibodies by them. Several species of Streptomyces from soil produces antibacterial and antifungal antibiotics. Most of the commercial antibiotics such as streptomycin, chloramphenicol, Terramycin and cyclohexamide have been produced from the mass culture ofStreptomyces. Thus, species of Streptomyces are the largest group of antibiotic producer’s in soil. The bacterial genus Bacillus produces an antifungal agent which inhibits growth of several soil fungi.

Antibiosis may result from a variety of other conditions operative in mixed populations. Certain fungi produce cyanide in concentrations toxic to other microorganisms and the algae elaborate fatty acids which exhibit a marked antibacterial activity. Many soil microorganisms, for example the myxobacteria and streptomycetes are antagonistic because they secret potent lytic enzymes which destroy other cells by digesting their cell wall or other protective surface layers. It appears that in the natural environment producers of lytic substances are often found in close proximity with sensitive organisms and do not predominate over them.

Ammensalism is the interaction between two species, where one species suppresses the growth of other by producing toxins like antibiotics or harmful gases like methane, ethylene, nitrite, or HCN or sulphides and other volatile sulphur compounds.

**Competition**

Soil is inhabited by different kinds of microorganisms, and therefore they exhibit competition among themselves for nutrients and space. In this kind of situation, the best adapted microorganism will predominate or eliminate the others which are dependent upon the same limited nutrient substance. The organisms with inherent ability to grow fast are better competitors.

Exogeneous nutrients are required for the germination of chlamydospores of Fusarium, Oospores of Aphanomyces and conidia of Verticillium dahlaein soil. But other fungi and soil bacteria deplete these critical nutrients required for spore germination and thereby hinder the spore germination resulting into the decrease in population. Soil bacteria compete for space and suppress the growth of the fungal population.

**Parasitism**

Parasitism is the relationship between two organisms, in which one organism lives in or on another organism. The parasite is dependent upon the host and feeds on the cells, tissues or fluids of the host organism. The parasite lives in intimate physical contact with the host and forms metabolic association with the host. All major groups of plants, animals, and microorganisms are susceptible to attack by microbial parasites.

The bacterial parasite of Gram-negative bacteria Bdellovibrio bacteriovorus which is widespread in soil and sewage attaches to a host cell at a special region and eventually causes the lysis of that cell. As a consequence, plaque like areas of lysis appear when these parasites are plated along with their host bacteria. Parasitism is widely spread in soil communities. Viruses which attack bacteria (bacteriophages), fungi, and algae are strict intracellular parasites since they cannot be cultivated as free-living forms. There are also many strains of fungi which are parasitic on algae and other fungi by penetration into the host. Fungi with antagonistic activity toward plant pathogens have an essential role in plant growth and health. Mycoparasites and presumptive mycoparasites have biocontrol potential, some are responsible for natural suppressiveness of soils to certain plant pathogens. Several species of Trichoderma were used successfully against certain pathogenic fungi. Trichoderma sp. was used as commercial bio-fungicides to control a range of economically important soil-borne fungal plant pathogens. Soils contain a large number and great diversity of oospore parasites, which may have the potential to reduce populations of plant pathogenic Phycomycetes in soil.

**Predation**

Predation is an association in which predator organism directly feed on and kills the pray organism. Predators may or may not kill their prey prior to feeding on them, but the act of predation often results in the death of its prey and the eventual absorption of the prey's tissue through consumption.

Many species of the soil-dwelling myxobacteria are predators of other microbes. Many myxobacteria, e.g., Myxococcus xanthus, exhibit several complex social traits, including fruiting body formation and spore formation cooperative swarming with two motility systems, and group predation on both bacteria and fungi. Myxobacteria use gliding motility to search the soil matrix for prey and produce a wide range of antibiotics and lytic compounds that kill and decompose prey cells and break down complex polymers, thereby releasing substrates for growth. The nematophagous fungi are the best predatory soil fungi. Species of Arthrobotrytis and Dactylella are known as nematode trapping fungi.

**Rhizobium-legume symbiosis, a paradigm in plant-bacteria interactions**

Rhizobia are able to establish mutualistic nitrogen- fixing symbioses with legume plants. These bacteria use nitrogenase, an exclusive prokaryotic enzyme to reduce molecular nitrogen into ammonia, to fulfil the host’s nitrogen nutritional needs. In exchange, bacteria are provided with an exclusive ecological niche (the nodules) where they can multiply at the expense of plant carbohydrates. The formation of nitrogen-fixing nodules requires the mutual secretion and correct recognition of several signal molecules by both the plant and the bacteria however, the process is still not fully understood. The best known strategy used by rhizobia to establish symbiosis with legume plants involves the production of lipochitooligosaccharidic Nod factors (NFs) in response to specific flavonoids excreted by the plant. Nod factors induce several responses in the plant which are essential for rhizobial infection and nodule organogenesis such as curling of the root hairs and the formation of nodule primordia after the activation of cortical cell division. Bacteria attached to root hairs penetrate the root through a tubular structure called the infection thread, which grows towards the root cortex where the nodule primordium develops. When the infection thread reaches the primordium, the bacteria are released into the plant cell cytoplasm where they differentiate into endosymbiotic forms, known as bacteroids. Particularly intriguing is how the plant is set to alter its physiology and root anatomy to gain access to nitrogen fixation, a process that will be donated by an intruder only after nodule development and bacterial infection are correctly achieved. As outlined below, some of the signals and the associated responses resemble, either structurally and/or functionally, many of those involved in pathogenic interactions.

Rhizobial infection in legumes triggers several plant responses that resemble those observed in plants challenged with pathogenic bacteria. Cytological and biochemical features of HR have been observed in the legume-rhizobia interaction associated to aborted infection threads, that is interpreted as part of a mechanism called autoregulation of nodulation that allows the plant to control nodule number. Accumulation of salicylic acid (SA), a phenolic compound that plays a key role in plant defence, has been observed in legume plants after inoculation with incompatible rhizobia. The production of the specific NFs prevents accumulation of SA that otherwise would inhibit nodule formation. Production of reactive oxygen species (ROS) upon plant perception of avirulent pathogens have several roles directed towards confinement of the infective microbes including the killing of microbes, reinforcement of cell walls and induction of defence gene expression. ROS also accumulate during the *Rhizobium*-legume interaction but depending on the intensity and localization of the oxidative burst the ROS could have dual roles. The first role is as part of a typical defence reaction to limit bacterial entry and secondly, as compounds needed for infection thread progression or even as signals for the expression of plant and/or bacterial symbiotic genes.

It seems clear that legumes and non-legumes have similar perception systems and protective responses against the infection by microbes. It is critical then that the establishment of any kind of compatible plant–bacteria association requires the microorganisms to evade detection or avoid host defenses. It is also exciting that both mutualistic and pathogenic bacteria seem to use similar strategies and weapons to elude or modulate the plant's battery of resources directed to arrest bacterial invasion. Cell-cell communication through quorum sensing (QS) is essential to coordinate within a bacterial population the expression of genes important for the colonization and infection of the host. Deficiencies in QS lead to the reduction of virulence in phytopathogens and to altered nodulation and nitrogen fixation by rhizobia. Quorum sensing is involved in the transition from a free-living to a plant-interacting lifestyle, by turning off behaviours like motility and activating others such as the production of surface polysaccharides (SPSs), biofilm formation or secretion of proteins needed for the successful invasion of the host, both by mutualistic and pathogenic bacteria. Some of those components, like type III and type IV protein secretion systems are needed for the injection of secreted proteins that interfere with plant physiology and metabolism to modulate host defences. Surface polysaccharides can have multiple roles such as protecting the bacterial cell from antimicrobial compounds such as ROS that are released by the host or by participating in the suppression of host defence reactions. The importance of antioxidant systems, involving catalases and superoxide dismutases as virulence factors of some phytothogenic bacteria correlates with the important role of these detoxifying bacterial enzymes for the establishment of the *Rhizobium*-legume symbiosis. Therefore, the

*Rhizobium*-legume symbiosis can be considered a model system that can provide new insights about molecular mechanisms that could also be important for different plant-bacteria interactions.

**Plant antimicrobial peptides in pathogenic and mutualistic interactions**

Part of the plant immune system relies on the production of antimicrobial peptides (AMPs) like defensins, thionins and lipid transfer proteins. AMPs are ribosomally synthesized antibiotics produced by nearly all organisms, from bacteria to plants and animals. AMPs include all peptides that can kill microbes but not those that exhibit a hydrolytic activity, such as lysozymes, chitinases and glucanases. Certain AMPs exhibit a narrow spectrum, while others are active against a broad-spectrum of microbes like Gram-negative and Gram-positive bacteria and fungi. The peptides can be membrane-disruptive resulting in cell lysis, or may also be actively taken up by transporters to reach their intracellular targets. They bind DNA, RNA and proteins and inhibit cell wall, DNA, RNA or protein synthesis . Most plant AMPs are characterized by typical arrangements of cysteine residues and belong to a large group of small Cysteine- Rich Peptides (CRPs). This abundance of AMP-like genes suggests that plants have a broad repertoire of AMPs to fight pathogens, but also the capacity to evolve towards new AMPs with novel specificities.

Very recently, legume AMPs have been revealed to be essential for *Rhizobium*-legume symbiosis. Inside the symbiotic nodule cells, the rhizobia become capable of reducing atmospheric nitrogen to ammonium only after differentiation into bacteroids. These are differentiated bacteria with altered physiology and metabolism. In legumes forming indeterminate nodules, like the model plant *Medicago truncatula*, bacteroids are characterized by their elongated or branched morphologies and show amplified genome content and increased membrane permeability. These bacteroids are incapable of cell division and thus are irreversibly differentiated, non- cultivable bacteria. This terminal differentiation of bacteroids is not observed in all legumes and therefore is not essential *per se* for symbiotic nitrogen fixation, but it could improve the symbiotic efficiency of the bacteroids. It has been recently shown that *M. truncatula* controls rhizobial bacteroid differentiation through the production of nodule-specific AMPs of the Nodule-specific Cysteine-Rich peptides (NCR) family. These NCR peptides are targeted to the bacteria and enter the bacterial membrane and the cytosol. A rhizobial protein BacA, also present in an endosymbiotic pathogen *Brucella*, might be required for uptake of these peptides. Thus, it seems that legumes such as *M. truncatula* have been able to evolve AMPs effectors of the innate immune system to manipulate their endosymbionts in order to maximize their own profits. This represents an extraordinary and clear example of how a typical plant defence response, production of antimicrobial peptides, has been adapted to control the proliferation of the invading microbe but also to obtain a benefit from the intruder.

**Nematode-Killing Fungi**

Plant-parasitic nematodes cause severe damages to world agriculture every year. Similarly, animal-parasitic nematodes infect grazing animals such as sheep and can kill the host animal, leading to significant financial loss for the farmers. Treatment is almost exclusively with chemicals, nematicides that kill the worms. However, nematodes are now becoming resistant to the chemicals and also causing significant environmental pollutions. In recent years, nematophagus fungi, one of the natural enemies of nematodes, have been proposed as biological agents to control the harmful nematodes because of they have ability to infect and kill these nematodes. Nematophagus fungi include a wide and diverse range of fungi that can antagonize nematodes. More than 150 species of fungi are known to attack nematodes or their eggs. According to the different pathogenic mechanisms nematophagus fungi can be classified into three categories:

**Parasitic fungi** which produce spores that infect nematodes.
**Nematode-trapping fungi** which catch their prey with specialized hyphal devices.
**Toxic fungi** which produce toxin.

**Parasitic Nematophagus Fungi**

These fungi are obligate parasite and thus spend almost all their life cycle inside infected nematodes and only emerge to sexually reproduce and disperse their spores. Parasitic fungi infect nematodes mainly by ingestive spores (*Harposporium spp.*) or adhesive spores (*Drechmeria coniospora*). They use the nematode as their only food source. The spores release a chemical which attracts the nematodes towards it as potential food. This helps to ensure that attachment takes place. The fungal spores are either directly ingested by the nematode, where they attach to the gut cuticle or are sticky and adhere to the external cuticle of nematode. Once attached, the spore germinates, producing a germ tube which penetrates the nematode's cuticle. The fungus grows and creating a network of hyphae that produce enzymes which breakdown the nematode. The fungus generates aerial hyphae along the length of the dead nematode. These break through the cuticle and produce aerial spores which are dispersed by the wind and rain. If conditions are not favorable, the fungus can produce resistant spores within the dead free- living nematodes. They produce traps at intervals along the length of their hyphae that capture, penetrate, kill and digest a nematode's contents. The traps are usually formed in response to the presence of substances produced by the nematodes. Some endoparasites, for example, *Catenaria anguillulae* can produce zoospores that are attracted to nematodes before adhesion. The attachment is followed by encystment on the cuticle surface.

**Nematode-Trapping Fungi**

Nematode-trapping fungi are facultative parasites. The primary function of predatory fungi is wood decay. Wood is mainly composed of carbohydrates-cellulose and lignocellulose. Wood has very high carbon: nitrogen (C:N) ratio. Most organisms require a C: N ratio of 30: 1 to produce nucleic acids, proteins and enzymes for their good growth. For predatory fungi nitrogen is the limiting factor for growth. Nematode- trapping or predatory nematophagus fungi get their extra nitrogen from digesting the nematode's biomass. Thus, predatory fungi have two phases–the predatory parasitic phase and the saprotrophic phase that run in parallel to supply them with the correct nutrients for growth.

Over 200 species of fungi use specialized structures, called traps to capture nematode. Nematode-trapping fungi include *Arthrobotrys* oligospors, *Monoacrosporium haptotylum* and many more The type of trap produced will depend on the fungal species involved. The predatory fungus secretes chemicals that attract the nematode towards it by chemotaxis, leading quickly to its death. Two basic trapping mechanisms have been observed in carnivorous fungi that are predatory on nematodes-constricting rings (active traps) and adhesive structures (passive traps).

**Constricting Rings**

This is the most sophisticated of the trapping devices and is common in the species *Drechslerella*. The nematode wriggles into the ring hoping to find food, but as it touches the ring it triggers a response. Three curved cells at the end of a short stalk, which make up the closed loop, swell rapidly inwards, and immediately crush the worm. Once ensnared, the fungus pierces the nematode's cuticle using a narrow penetration peg, which swells inside the host to form an infection bulb that the hyphae grow from. Fungal enzymes breakdown the contents of the nematode and the nutrients are translocated within the hyphal system for growth or spore production.

**Adhesive Traps**

Nematode-trapping fungi form different nematode-trapping devices that include adhesive hyphae, adhesive networks, adhesive knobs or branches, and non-adhesive rings. Adhesive traps capture their prey by means of an adhesive layer covering all or part of the trap. The adhesive on the fungal trap binds strongly to sugar compounds on the surface of the nematode. Different kinds of adhesive traps include: Networks resemble a mesh of interlocking loops which ramify through the soil. It is the most common type of adhesive trap (e.g., *Arthrobotrys oligospora*). Knobs are erect stalks with an adhesive bulb at the end that are spaced out along the length of the hyphae (*Deuteromycetes and Basidiomycetes*).

**Non-constricting rings**

Non-constricting rings – composed of three cells that do not change in size or shape. Fungi produce a

dhesive knobs often produce these rings as an alternative trapping device (Dactylellina candidum). The traps have extensive layers of extracellular polymers which have been considered important for the attachment of the traps to nematode surfaces. As with other pathogens, the nematode- trapping fungi enter into the host through both enzyme degradation and mechanical pressure. Several extracellular hydrolytic enzymes including serine proteases, collagenase, chitinase and other hydrolytic enzymes (such as having lipolytic activity) are key virulence factors involved in the penetration process. After penetration, the hosts will be eventually degraded by the invading fungi.

**Symbiotic luminescent bacteria**

Bioluminescence, which is the natural production of light by organisms, occurs in animals, fungi, protests and certain bacteria. Light is produced when the compound luciferin is oxidised. Larger organisms such as squid contain light organs where light production takes place, in smaller organisms such as bacteria, the whole organism luminesces.

Over 90% of species which inhabit depths greater than 700m, are thought to possess the ability to bioluminescence. Due to its diversity and phylogenetic distribution, it is thought that bioluminescence may have evolved independently up to 30 times over the course of evolution. Rees *et al*. (1998) proposes that the luminescent substrates (luciferins) of the luminous reactions are the evolutionary core of most systems. Rees *et al*. (1985) also suggests that oxygen detoxifying mechanisms provide excellent foundations for the emergence of many bioluminescent systems.

In most species, luminescence is produced by the animal, however in a minority, which will be the subject of this essay; the light is produced by bacteria which function as symbionts of the animal within its light organs. Bioluminescent symbiotic associations with luminous bacteria have been identified in species of teleost fish and species of loliginid and sepiolid squids. The occurrence of bioluminescent symbiosis in pyrosomes and salps is still an unresolved issue.

The host animals utilise the luminescence for a variety of reasons. These include: predator avoidance (through counter-illumination and flashing), mating related signalling, attracting and locating prey and navigation in dark or low-light environments. Claes and

Dunlap 2006 noted that the bioluminescent symbiosis between luminous bacteria and their hosts is a unique type of symbiosis in that the metabolic benefit for the host is light for display purposes as opposed to a nutrient for growth purposes.

Of the luminous bacteria, four species have been identified in bioluminescent symbiotic associations. They are *Vibrio fischeri*, *V. logei*, *Photobacterium leiognathi* and *P. phosphoreum* . Genetic sequencing of bacteria involved in bioluminescent symbioses of ceratioid fish suggests that these fish contain as yet unidentified species of luminous bacteria. Luminous bacteria in the marine environment have been shown to be extremely resourceful when adapting to a varied selection of niches, including acting as saprophytes on faecal material, and pathogenic associations with marine mammals.

**Bioluminescence Symbioses, Mechanisms and Significance in Squid**

Bioluminescent squid species contain at least three species of luminous bacteria in symbiotic associations. Sepiolid squids contain *Vibrio fischeri* and *V. logei* while Loliginid squids contain *Photobacterium leiognathi*. In squids, the light organs where the symbiotic associations occur are bilobed organs found ventrally within the mantle cavity, adjacent to the ink sac.

Squids acquire their symbiotic luminous bacteria by horizontal transfer from the surrounding seawater. Based on studies on *Euprymna scolopes*, Wei and Young (1989) suggested that each generation of squid acquire infection from free living bacteria rather than from the egg. Most hatchlings produce light within 24 h. The bacteria must be motile to complete this transfer as a barrier of mucus and/or a current generated by cilia movement is present entering the light organ. Studies also show that the light organ habitat in *E. scolopes* is one of such specifity that only *V. fischeri* can survive. However, studies by Fidopiastis *et al*. (1998) revealed two separate species of luminous bacteria (*V.*

*fischeri* and *V. logei*) co-occurring within the light organs of *Sepiola spp*. Temperature may be a factor influencing this, with warmer temperatures favouring *V. fischeri* and colder temperatures favouring *V. Logei*.

Squid release their symbiotic bacteria into the surrounding water; in the case of *E. scolopes*, every 24 h resulting in locations which contain hosts becoming extremely enriched with the bacteria. Bacteria populations are thought to double at least once and possibly more every 24 h. Initiation of future associations may be dependant on this exudation of bacteria. In *E. scolopes*, this generally takes place at dawn or first illumination. These light organ dynamics are a micro-ecology, functioning possibly to continually select more composite strains of symbiotic bacteria.

Symbiosis appears to be the normal state of *E scolopes*, starting immediately after hatching. However, it is not tested whether the absence of a light organ would affect its survival rate.

Another aspect of the squid – bacteria symbiotic association is the morphogenesis of the light organ due to the presence of certain bacteria. Studies by Doino and McFall-Ngai (1995) on *E. scolopes* and *V. fischei* showed that when the squid was exposed to the symbiotic bacteria for 12h, tissue regression in the light organ took place over the next few days. This morphogenesis consists of lateral ciliated epithelial appendages coalescing in the light organ into a ciliated duct.

**Bioluminescence Symbioses, Mechanisms and Significance in Fish.**

Bioluminescent fish species contain at least three identified species of luminous bacteria in symbiotic associations; *Vibrio fischeri*, *Photobacterium leiognathi* and *P. phosphoreum*. The symbiotic bacteria are extra cellular and inhabit tubules and canals of the fish's light organ. The fish keep a high density of bacteria present in their light organ, a factor which leads to continuous illumination of the organ. This is due to the synthesis of luciferase in fish necessitating a specific concentration of auto-inducer emission by the bacteria.

The light organ in fish may be located in various places. Development of the light organ in some fish (*Monocentris japonicus*) may require first the acquiring of symbionts (*V. fischeri*). As with squid, fish also acquire their luminescent symbionts through horizontal transfer from the surrounding seawater. Studies by Wada *et al*. (1999) of *Leiognathus nuchalis* demonstrated that the fish's offspring are without symbiotic bacteria (aposymbiotic) at hatching and require the presence of adult fish to gain symbionts, with offspring being kept apart from adult fish failing to acquire luminescence. This is due to the exudation of bacteria into the surrounding seawater from the adults. Luminescence occurs in offspring kept with adults mostly within 48 h of hatching. It is also necessary that the symbiotic bacteria are motile for this to occur.

Specificity of the *P. leiognathi* / Leiognathid fish symbiosis is maintained at the species level of the bacteria as opposed to the individual level. Symbionts in any one host are a homogenous culture and completely distinctive to another individual host of the same species. Studies by Nealson *et al*. (1984) on the luminous fish *Cleidopus gloriamaris* and *Photoblepharon palpebratus* showed that the fish also exude luminous bacteria. Monocentrid fish, such as *M. japonicus* release bacteria directly into the seawater, whereas Leiognathid fish such as *L. nuchalis* release via the gut tract into the seawater

Studies on bioluminescent symbiotic associations between *P. fischeri* and *M. japonica* have shown bacteria to produce greater luminescence under conditions of low oxygen and low growth. Studies by Dunlap (1984) on *P. leiognathi* in species of Leiognathid fish compared bacteria *in situ* (light organ) and bacteria cultured *in vivo*. This showed major differences such as lack of motility and flagella *in situ* and 20 to 30 times slower growth *in situ*, which reiterates the minimal growth, maximum luminescence model.

As with squid, there may be a system of controlling the density of bacteria in Leiognathid fish in order to maximise luminescence. This is also thought to be through the autoinducer mechanism. It is proposed that *P. phosphoreum* and *V. fischeri* metabolise glucose to pyruvate which is used in respiration by mitochondria which decreases oxygen and increases luminescence. Controlling the amount of carbon reaching bacteria forces the bacteria to use the luminescence system as an alternative respiratory pathway (to re-oxidise reduced co-enzymes).

**Significance of Bioluminescence Symbioses.**

Bioluminescence symbioses are highly significant examples of symbioses in the marine environment mainly due to their ecological processes. Bioluminescence is a widespread trait in the marine environment, particularly at depth, however in the vast majority of organisms, this luminescence is self produced. One significant aspect of bioluminescent symbioses is the small minority of species it occurs in.

Perhaps the most significant aspect of bioluminescent symbioses is its contrast with other symbioses within the marine environment with regard to its functions. In most bacteria-animal symbiotic associations, the host animal benefits from the association through the acquiring of nutrients (e.g. through nitrogen fixation by the bacteria) which are used for growth purposes by the host. However, in bioluminescence symbioses, the host benefits through the acquisition of light, which is used for a variety of purposes, not directly linked with growth. In squid, these may be to camouflage or to deter other organisms through fright. In fish, these may be to attract predators, become invisible and to communicate. The fish/squid in turn provide the bacteria with nutrients, oxygen and shelter within the light organ. In fact, benefits to symbiotic luminous bacteria still remain unclear.

Another significant ecological aspect of bioluminescent symbiotic associations is the extent of influence the host animal has on the bacterial symbionts, which is rather great. In studies on bioluminescent symbioses in squid, Lee and Ruby (1994) noted that the abundance and distribution of luminous marine bacteria is driven primarily by its symbiotic association with the animal host. The host animals exert an influence on the bacteria in a number of ways. The low growth-high luminescence model, mentioned earlier represents a very significant control on the bacteria as the host controls the supply of oxygen and/or nutrients to the bacteria. Also, the exudation of bacteria from the light organs of squid and fish as controls the size, density and distribution of the bacteria populations through wide dispersal. Tight crowding leads to a lack of motility and flagella in bacteria within the light organ compared to bacteria in culture.

Of perhaps less significance is the influence bacteria exert on their hosts. Claes and Dunlap (2000) found that the squid *E. scolopes* survived just as well without its luminous symbionts as animals with theirs. This is in contrast to most other bacterial symbiotic associations in the marine environment, where the bacteria would have a much greater influence on the host as the host would be nutritionally dependant on the bacteria. The bacteria do however in some cases contribute to the developpment of the light organ through morphogenesis as Yamada *et al*. (1979) proposes that light organ development in monocentrid fish requires the presence of bacteria while Claes and Dunlap (2000) report that development of the light organ in squid is not reliant on symbionts being present.

**ENVIRONMENTAL MICROBIOLOGY - 18MBU403**

**Possible Questions – Unit II**

1. Write in detail about the structure and functions of ecosystem
2. Describe in detail about Extremophiles
3. Write a note on symbiotic and non-symbiotic interactions
4. Briefly explain microbe – animal interactions
5. Write a brief notes on carbon cycle
6. Briefly explain about elemental i.e. iron and manganese cycle
7. Give an account on negative microbial interaction
8. Write a note on the bioluminescence symbiosis.
9. Write a note on mutualism and synergism.

| Sno | Question | Option 1 | Option 2 | Option 3 | Option 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | ______ are aerobic and free-living nitrogen nitrogen fixers | *Frankia* & *Azospirillum* | *Clostridium*& *Desulfovibrio* | *Beijerinckia* & *Klebsiella* | *Rhizobium*& *Anabaena* | *Beijerinckia* & *Klebsiella* |
| 2 | ______ are genes encoding enzymes involved in the fixation of atmospheric nitrogen | *mif* | *nif* | *sif* | *nod* | *nif* |
| 3 | ________ catalyze conversion of atmospheric nitrogen to ammonia | Kinase | Hydrogenase | Nitrogenase | Phosphatase | Nitrogenase |
| 4 | ________ is a typical example of symbiotic nitrogen fixation seen in paddy fields | Azolla-*Anabaena* | Alder-*Frankia* | Legume-*Rhizobium* | Higher plants-*Mycorrhizae* | Azolla-*Anabaena* |
| 5 | _________ recycles the $H_2$ produced during $N_2$ fixation, thereby minimizing the loss of energy | Reductase | Catalase | Nitrogenase | Hydrogenase | Hydrogenase |
| 6 | A free-living anaerobic photosynthetic bacterium | *Anabaena azollae* | *Clostridium thermocellum* | *Rhodospirillum rubrum* | *Klebsiella pneumoniae* | *Rhodospirillum rubrum* |
| 7 | A free-living soil bacteria that is involved in nitrogen fixation | *Alcaligenes* | *Acetobacter* | *Pseudomonas* | *Azotobacter* | *Azotobacter* |
| 8 | Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biological nitrogen fixation | 8 | 16 | 32 | 64 | 16 |
| 9 | Apart from biological nitrogen fixation by | Cyclone | Thunder | Raining | Lightning | Lightning |

| | | | | | | |
|---|---|---|---|---|---|---|
| | microbes, __________ can fix atmospheric nitrogen | | | | | |
| 10 | Bacteria that forms root nodules in legume plants | *Rhizobium* | *Azotobacter* | *Azospirillum* | Cyanobacteria | *Rhizobium* |
| 11 | Biological nitrogen fixation was discovered by | Winogradsky | Beijerinck | Pasteur | Koch | Beijerinck |
| 12 | Chemicals produced by the Rhizobia called _________ that cause the colonized root hairs to curl | Pod factors | Nod factors | Sod factors | Mod factors | Nod factors |
| 13 | Example of associative nitrogen fixation | Legume-*Rhizobium* | Rice-*Azospirillum* | Higher plants-Mycorrhizae | Azolla-*Anabaena* | Rice-*Azospirillum* |
| 14 | *Frankia* is a | Bacteria | Actinomycete | Fungi | Algae | Actinomycete |
| 15 | Group of irregularly shaped bacteria in root nodules are called as | Bacteroids | Asteroids | Mesteroids | Histeroids | Bacteroids |
| 16 | In biological nitrogen fixation, ____ moles of ammonia are produced from one mole of nitrogen gas | 2 | 4 | 6 | 8 | 2 |
| 17 | In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as | Cyanocysts | Nitrocycts | Heterocysts | Homocysts | Heterocysts |
| 18 | In root nodules, ____________ bind and regulate the levels of oxygen in the nodule | Teghemoglobin | Peghemoglobin | Leghemoglobin | Hemoglobin | Leghemoglobin |
| 19 | Legume plants belongs to | *Solanaceae* | *Rosaceae* | *Astraceae* | *Fabaceae* | *Fabaceae* |
| 20 | Most abundant gas in atmosphere | Nitrogen | Oxygen | Carbon dioxide | Hydrogen | Nitrogen |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21 | Nitrogenase enzyme consists of | Iron protein | Molybdenum-iron protein | Iron protein and a molybdenum-iron protein | Hemoglobin | Iron protein and a molybdenum-iron protein | |
| 22 | Rhizobia are attracted to ___________ released by the host legume's roots | Flavonoids | Enzymes | Toxins | Chemicals | Flavonoids | |
| 23 | The enzyme nitrogenase is inhibited by | $CO_2$ | Sulfur | Hydrogen | Oxygen | Oxygen | |
| 24 | Which is not true about *Anabaena* and *Nostoc* | Filamentous | Nitrogen fixing | Cyanobacteria | Symbiotic | Symbiotic | |
| 25 | The majority of hydrogenases in prokaryotes are ________ containing enzymes | Nickel | Copper | Molybdenum | Sulfur | Nickel | |
| 26 | With associative nitrogen fixation, which one of the following genera is associated? | Azotobacter | Escherichia | Rhizobium | Anabena | Azotobacter | |
| 27 | The conversion of nitrogen to ammonia or nitrogenous compounds is called as_______ | Nitrogen assimilation | Nitrogen fixation | Denitrification | Nitrification | Nitrogen fixation | |
| 28 | The organisms which is not symbiotic nitrogen fixing cyanobacteria_______ | Anthoceros | Azolla | Cycas | Gnetum | Gentum | |
| 29 | All the following are free living nitrogen fixers except_________ | Rhizobium | Azotobacter | Rhodospirillum | Clostridium | Rhizobium | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 30 | Anabena is a nitrogen fixer present in the root pockets of________ | Marselia | Salvinia | Pistia | Azolla | Azolla |
| 31 | Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by __________ | hydrogenase | nitrogenase | dinitrogenase | nitrate reductase | nitrogenase |
| 32 | Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere? | Bacteria | Algae | Nematodes | Moulds | Bacteria |
| 33 | A major plant macronutrient found in nucleic acids and proteins is __________ | calcium | nitrogen | sulphur | iron | nitrogen |
| 34 | Organisms capable of converting nitrogen to nitrate are________ | yeast | bacteria | roundworms | moulds | bacteria |
| 35 | Conversion of nitrite to nitrate is carried out by ________ | Nitrosomonas | Nitrosococcus | Nitrobacter | Clostridium | Nitrobacter |
| 36 | The nonsymbiotic bacteria which fix nitrogen live in the soil independently are | Azotobacter | Anabena | Rhizobium | Azolla | Azotobacter |
| 37 | Which of the following is not the biofertilisers producing bacteria? | Nostoc | Anabaena | Both (a) and (b) | Clostridium | Clostridium |
| 38 | Which of the following is capable of oxidizing sulfur to sulfates? | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Thiobacillus thiooxidans |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 39 | Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrotrophic bacteria. The reasons may be due to | slow growth | medium growth | fast growth | no growth | slow growth | |
| 40 | _______ play a key role in the transformatio nof rock in the transformation of rock to soil | Cyanobacterium | pectin decomposing bacteria | denitrifying bacteria | | Cyanobacteria | |
| 41 | Denitrification may be distinguished as | dissimilative and assimilative | assimilative | Partially dissimilative | Partially assimilative | Dissimilative and assimilative | |
| 42 | All of the following are examples of negative symbiosis________ | amensalism | competition | commensalism | parasitism | competition | |
| 43 | The reservior for nitrogen is __________ | the atmosphere | rocks | ammonia | nitrates | the atmosphere | |
| 44 | Most soil protozoa are flagellates or amoebas, having their dominant mode of nitrogan as | Ingestion of bacteria | ingestion of mold | ingestion of fungi | ingestion virus | ingestion of bacteria | |
| 45 | Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrotrophic bacteria. The reasons may be due to | medium growth | fast growth | Good growth | slow growth | slow growth | |
| 46 | An example of a symbiotic nitrogen fixer is________ | Azotobacter | Beijerinckia | Clostridium | Rhizobium | Rhizobium | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 47 | In the process of nitrogen fixation, which of the following microorganism is involved? | Non symbiotic microorganisms only | Symbiotic microorganisms only | Non symbiotic and symbiotic microorganisms only | Symbiosis | Non symbiotic and symbiotic microorganisms only | |
| 48 | The physical structure of soil is improved by the accumulation of _______ | mold mycelium | minerals | water | metals | Mold mycelium | |
| 49 | ________ play a key role in the transformation of rock to soil | Cyanobacteria | Pectin decomposing bacteria | Nitrifying bacteria | De-nitrifying bacteria | Cyanobacteria | |
| 50 | The conversion of molecular nitrogen into ammonia is known as_______ | nitrification | denitrification | nitrogen fixation | ammonification | nitrogen fixation | |
| 51 | Some microorganisms have the ability to increase the nitrogen content of soils, are called as | Nitrogen fixation | denitrification | nitrification | ammonification | nitrogen fixation | |
| 52 | Which of the following soil microorganism is involved in the reduction of sulfates to H2S | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Desulfotomaculum | |
| 53 | Which of the following fungi on infecting crop roots can improve their uptake of phosphorus and other nutrients? | Saccharomyces cerevisiae | VA Mycorrhiza | Candida torulopsis | Aspergillus niger | VA Mycorrhiza | |

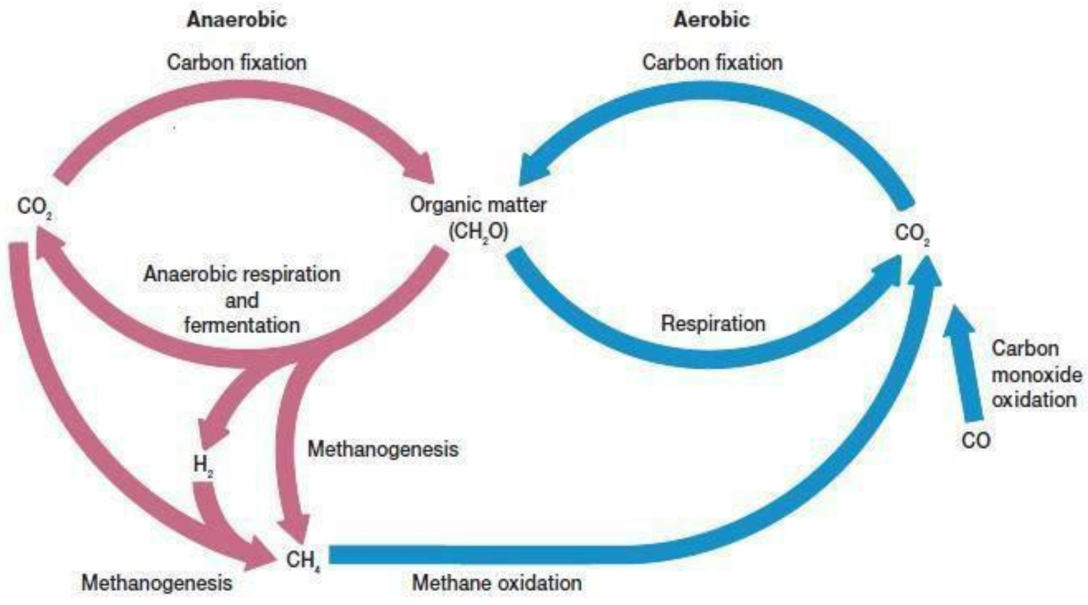
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 54 | Syntrophism involves | exchange of nutrients between two species | exchange of nutrients among species | no exchange of nutrients between two species | no exchange of nutrients among species | exchange of nutrients between two species | |
| 55 | Assimilative denitrification is done by ________ | Plants | animals | virus | protozoans | plants | |
| 56 | The diagnostic enzyme for nitrogen-fixing organisms is | nitrogenase | nitrate reductase | nitrate oxidase | dehrogenase | nitrogenase | |
| 57 | Which of the following is the type of endosymbiosis | Commensalisms | cooperation | mutualism | predation | Commensalism | |
| 58 | Azolla is used as biofertilizer as it has ________ | Rhizobium | Cyanobacteria | Mycorrhiza | Large quantity of humus | Cyanobacteria | |
| 59 | In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules | Bradyrhizobium japonicum | Rhizobium leguminosarum | Sinorhizobium meliloti | Both a and b | Rhizobium leguminosarum | |
| 60 | Ammonia produced in the bacteriod needs to be transported to the plants through which one of the following membrane | lipid membrane | periplasmic membrane | symbiosome membrane | plasma membrane | symbiosome membrane | |

**Nutrient Cycling Interactions**

Microorganisms, in the course of their growth and metabolism, interact with each other in the cycling of nutrients, including carbon, sulfur, nitrogen, phosphorus, iron, and manganese. This nutrient cycling, called biogeochemical cycling when applied to the environment, involves both biological and chemical processes. Nutrients are transformed and cycled, often by oxidation-reduction reactions that can change the chemical and physical characteristics of the nutrients. All of the biogeochemial cycles are linked, and the metabolism-related transformations of these nutrients have global-level impacts.

**Carbon Cycle**

Carbon can be present in reduced forms, such as methane (CH4) and organic matter, and in more oxidized forms, such as carbon monoxide (CO) and carbon dioxide (CO2). The major pools present in an integrated basic carbon cycle are shown in figure. Reductants (e.g., hydrogen, which is a strong reductant) and oxidants (e.g., $O_2$) influence the course of biological and chemical reactions involving carbon. Hydrogen can be produced during organic matter degradation especially under anaerobic conditions when fermentation occurs. If hydrogen and methane are generated, they can move upward from anaerobic to aerobic areas. This creates an opportunity for aerobic hydrogen and methane oxidizers to function.



**The Basic Carbon Cycle in the Environment.** Carbon fixation can occur through the activities of photoautotrophic and chemoautotrophic microorganisms. Methane can be produced from inorganic substrates ($CO_2$ + $H_2$) or from organic matter. Carbon monoxide (CO)—produced by sources such as automobiles and industry—is returned to the carbon cycle by CO-oxidizing bacteria. Aerobic processes are noted with blue arrows, and anaerobic processes are shown with red arrows.

Methane levels in the atmosphere have been increasing approximately 1% per year, from 0.7 to 1.6 to 1.7 ppm (volume) in the last 300 years. This methane is derived from a variety of sources. If an aerobic water column is above the anaerobic zone where the methanogens are located, the methane can be oxidized before it reaches the atmosphere. In many situations, such as in rice paddies without an overlying aerobic water zone, the methane will be re- leased directly to the atmosphere, thus contributing to global atmo- spheric methane increases. Rice paddies, ruminants, coal mines, sewage treatment plants, landfills, and marshes are important sources of methane. Anaerobic microorganisms such as *Methanobrevibacter* in the guts of termites also can contribute to methane production.

Carbon fixation occurs through the activities of cyanobacte- ria and green algae, photosynthetic bacteria (e.g., *Chromatium* and *Chlorobium*), and aerobic chemolithoautotrophs. In the carbon cycle depicted in figure, no distinction is made between different types of organic matter that are formed and degraded. This is a marked oversimplification because organic matter varies widely in physical characteristics and in the biochemistry of its synthesis and degradation. Organic matter varies in terms of elemental composition, structure of basic repeating units, linkages between repeating units, and physical and chemical characteristics.. The degradation of this organic matter, once formed, is influenced by a series of factors. These include (1) nutrients present in the environment; (2) abiotic conditions (pH, oxidation- reduction potential, $O_2$, osmotic conditions), and (3) the microbial community present.

The major complex organic substrates used by microorganisms are summarized in table. Of these, only previously grown microbial biomass contains all of the nutrients required for microbial growth. Chitin, protein, microbial biomass, and nucleic acids contain nitrogen in large amounts. If these sub- strates are used for growth, the excess nitrogen and other minerals that are not used in the formation of new microbial biomass will be released to the environment, in the process of mineralization. This is the process in which organic matter is decomposed to release simpler, inorganic compounds (e.g., $CO_2$, $NH_4^+$, $CH_4$, $H_2$). The other complex substrates in table contain only carbon, hydrogen, and oxygen. If microorganisms are to grow by using these substrates, they must acquire the remaining nutrients they need for biomass synthesis from the environment; in the process of immobilization.

**Complex Organic Substrate Characteristics That Influence Decomposition and Degradability**

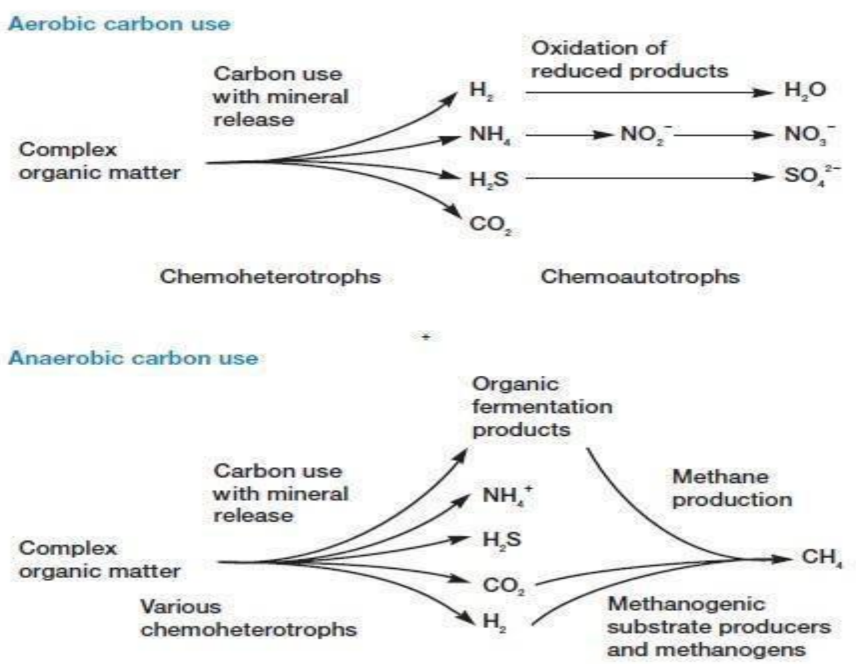
| | | | *Elements Present in Large Quantity* | | | | | *Degradation* | |
|---|---|---|---|---|---|---|---|---|---|
| **Substrate** | **Basic Subunit** | **Linkages (if Critical)** | **C** | **H** | **O** | **N** | **P** | **With $O_2$** | **Without $O_2$** |
| Starch | Glucose | α(1→ 4)<br>α(1→ 6) | + | + | + | − | − | + | + |
| Cellulose | Glucose | β(1→ 4) | + | + | + | − | − | + | + |
| Hemicellulose | C6 and C5 monsaccharides | β(1→ 4), β(1→ 3), β(1→ 6) | + | + | + | − | − | + | + |
| Lignin | Phenylpropane | C–C, C–O bonds | + | + | + | − | − | + | − |
| Chitin | *N*-acetylglucosamine | β(1→ 4) | + | + | + | + | − | + | + |
| Protein | Amino acids | Peptide bonds | + | + | + | + | − | + | + |
| Hydrocarbon | Aliphatic, cyclic, aromatic | | + | + | − | − | − | + | +− |
| Lipids | Glycerol, fatty acids; some contain phosphate and nitrogen | Esters | + | + | + | + | + | + | + |
| Microbial biomass | | Varied | + | + | + | + | + | + | + |
| Nucleic acids | Purine and pyrimidine bases, sugars, phosphate | Phosphodiester and *N*-glycosidic bonds | + | + | + | + | + | + | + |

The oxygen relationships for the use of these substrates also are of interest, because most of them can be degraded easily with or without oxygen present. The exceptions are hydrocarbons and lignin. Hydrocarbons are unique in that microbial degradation, especially of straight-chained and branched forms, involves the initial addition of molecular $O_2$. Recently, anaerobic degradation of hydrocarbons with sulfate or nitrate as oxidants has been ob- served. With sulfate present, organisms of the genus *Desulfovibrio* are active. This occurs only slowly and with microbial com-munities that have been exposed to these compounds for extended periods. Such degradation may have resulted in the sulfides that are present in “sour gases” associated with petroleum.

Lignin, an important structural component in mature plant materials, is a complex amorphous polymer based on a phenylpropane building block, linked by carbon-carbon and carbon-ether bonds. It makes up approximately 1/3 of the weight of wood. This is a special case in which biodegradability is dependent on $O_2$ availability. There often is no significant degradation because most filamentous fungi that degrade native lignin in situ can function only under aerobic conditions where oxidases can act by the release of active oxygen species. Lignin's lack of biodegradability under anaerobic conditions results in accumulation of lignified materials, including the formation of peat bogs and muck soils. This absence of lignin degradation under anaerobic conditions also is important in construction. Large masonry structures often are built on swampy sites by driving in wood pilings below the water table and placing the building footings on the pilings. As long as the foundations remain water-saturated and anaerobic, the structure is stable. If the water table drops, however, the pilings will begin to rot and the structure will be threatened. Similarly, the cleanup of harbors can lead to de- composition of costly docks built with wooden pilings due to in- creased aerobic degradation of wood by filamentous fungi. Rumen function provides a final example of the relationship between lignin degradation and oxygen. The rumen, being al- most free of oxygen, does not allow significant degradation of lignin present in animal feeds. The use of sugars and carbohydrates in the rumen leaves an inactive residue that can improve soils more effectively than the original feeds.

Patterns of microbial degradation are important in many habitats. They contribute to the accumulation of petroleum products, the formation of bogs, and the preservation of valuable historical objects.

The presence or absence of oxygen also affects the final prod- ucts that accumulate when organic substrates have been processed by microorganisms and mineralized either under aerobic or anaerobic conditions. Under aerobic conditions, oxidized products such as nitrate, sulfate, and carbon dioxide (figure) will result from microbial degradation of complex organic matter. In comparison, under anaerobic conditions reduced end products tend to accumulate, including ammonium ion, sulfide, and methane.



**The Influence of Oxygen on Organic Matter Decomposition.** Microorganisms form different products when breaking down complex organic matter aerobically than they do under anaerobic conditions. Under aerobic conditions oxidized products accumulate, while reduced products accumulate anaerobically. These reactions also illustrate commensalistic transformations of a substrate, where the waste products of one group of microorganisms can be used by a second type of microorganism.

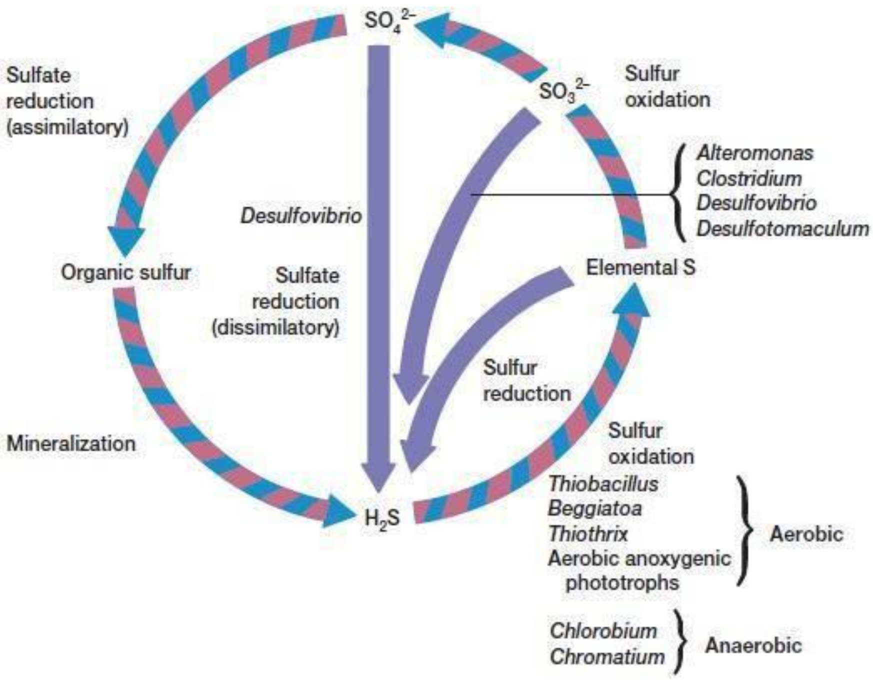
These oxidized and reduced forms, if they remain in the aerobic or anaerobic environments where they were formed, will usually only serve as nutrients. If mixing occurs, oxidized species might be moved to a more reduced zone or reduced species might be moved to a more oxidized zone. Under such circumstances, additional energetic possibilities (linking of oxidants and reductants) will be created, leading to succession and further nutrient cycling as these mixed oxidants and reductants are exploited by the microbial community.

**Sulfur Cycle**

Microorganisms contribute greatly to the sulfur cycle, a simplified version of which is shown in figure. Photosynthetic microorganisms transform sulfur by using sulfide as an electron source, allowing *Thiobacillus* and similar chemolithoautotrophic genera to function. In contrast, when sulfate diffuses into reduced habitats, it provides an opportunity for different groups of microorganisms to carry out sulfate reduction. For example, when a usable organic reductant is present, Desulfovibrio uses sulfate as an oxidant.

This use of sulfate as an external electron acceptor to form sulfide, which accumulates in the environment, is an example of a dissimilatory reduction process and anaerobic respiration. In comparison, the reduction of sulfate for use in amino acid and protein biosynthesis is described as an assimilatory reduction process. Other microorganisms have been found to carry out dissimilatory elemental sulfur reduction. These include *Desulfuromonas*, thermophilic archaea, and also cyanobacteria in hypersaline sediments. Sulfite is another critical intermediate that can be reduced to sulfide by a wide variety of microorganisms, including *Alteromonas* and *Clostridium*, as well as *Desulfovibrio* and *Desulfotomaculum*. *Desulfovibrio* is usually considered as an obligate anaerobe. Recent research, however, has shown that this interesting organism also respires using oxygen, when it is present at a dissolved oxygen level of 0.04%.

In addition to the very important photolithotrophic sulfur ox- idizers such as *Chromatium* and *Chlorobium*, which function under strict anaerobic conditions in deep water columns, a large and varied group of bacteria carry out aerobic anoxygenic photo synthesis. These aerobic anoxygenic phototrophs use bacteriochlorophyll *a* and carotenoid pigments and are found in marine and freshwater environments; they are often components of micro- bial mat communities. Important genera include *Erythromonas*, *Roseococcus*, *Porphyrobacter*, and *Roseobacter*. "Minor" compounds in the sulfur cycle play major roles in biology. An excellent example is dimethylsulfoniopropionate (DMSP), which is used by bacterioplankton (floating bacteria) as a sulfur source for protein synthesis, and which is transformed to dimethylsulfide (DMS), a volatile sulfur form that can affect atmospheric processes. When pH and oxidation-reduction conditions are favorable, several key transformations in the sulfur cycle also occur as the result of chemical reactions in the absence of microorganisms. An important example of such an abiotic process is the oxidation of sulfide to elemental sulfur. This takes place rapidly at a neutral pH, with a half-life of approximately 10 minutes for sulfide at room temperature.




**The Basic Sulfur Cycle.** Photosynthetic and chemosynthetic microorganisms contribute to the environmental sulfur cycle. Sulfate and sulfite reductions carried out by Desulfovibrio and related microorganisms, noted with purple arrows, are dissimilatory processes. Sulfate reduction also can occur in assimilatory reactions, resulting in organic sulfur forms. Elemental sulfur reduction to sulfide is carried out by Desulfuromonas, thermophilic archaea, or cyanobacteria in hypersaline sediments. Sulfur oxidation can be carried out by a wide range of aerobic chemotrophs and by aerobic and anaerobic phototrophs.

**Nitrogen Cycle**

Several important aspects of the basic nitrogen cycle will be dis- cussed: the processes of nitrification, denitrification, and nitrogen fixation (figure). It should be emphasized that this is a "ba- sic" nitrogen cycle. Although not mentioned in the figure, the heterotrophs can carry out nitrification, and some of these het- erotrophs combine nitrification with anaerobic denitrification, thus oxidizing ammonium ion to $N_2O$ and $N_2$ with depressed oxygen levels. The occurrence of anoxic ammonium ion oxidation (anammox is the term used for the commercial process) means that nitrification is not only an aerobic process. Thus as we learn more about the biogeochemical cycles, including that of nitrogen, the simple cycles of earlier textbooks are no longer accurate representations of biogeochemical processes.

Nitrification is the aerobic process of ammonium ion ($NH_4^+$) oxidation to nitrite ($NO_2^-$) and subsequent nitrite oxida- tion to nitrate ($NO_3^-$). Bacteria of the genera *Nitrosomonas* and *Ni- trosococcus*, for example, play important roles in the first step, and *Nitrobacter* and related chemolithoautotrophic bacteria carry out the second step. Recently *Nitrosomonas eutropha* has been found to oxidize ammonium ion anaerobically to nitrite and nitric oxide (NO) using nitrogen dioxide ($NO_2$) as an oxidant in a denitrifica- tion-related reaction. In addition, heterotrophic nitrification by bacteria and fungi contributes significantly to these processes in more acidic environments.

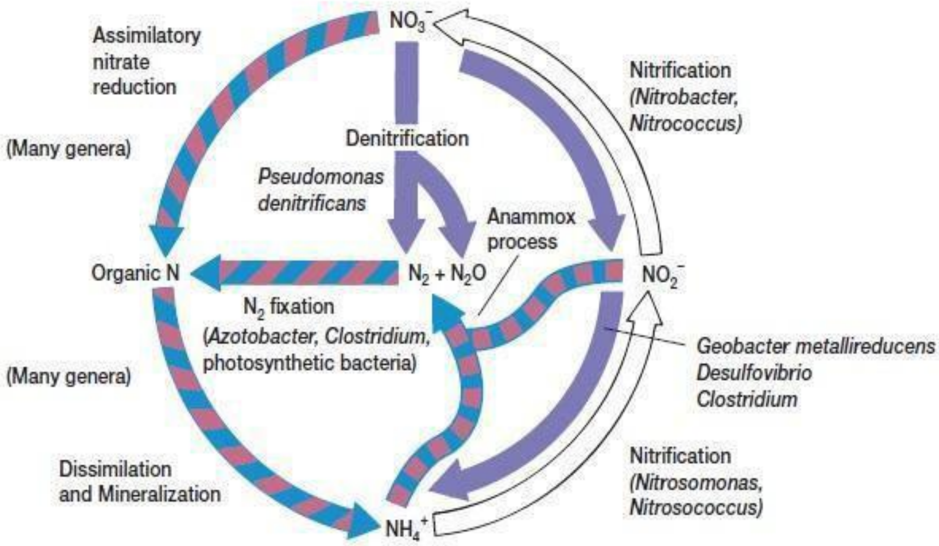
The process of denitrification requires a different set of envi- ronmental conditions. This dissimilatory process, in which nitrate is used as an oxidant in anaerobic respiration, usually involves heterotrophs such as *Pseudomonas denitrificans.* The major products of denitrification include nitrogen gas ($N_2$) and nitrous oxide ($N_2O$), although nitrite ($NO_2^-$) also can accumulate. Nitrite is of environmental concern because it can contribute to the formation of carcinogenic nitrosamines. Finally, nitrate can be transformed to ammonia in dissimilatory reduction by a variety of bacteria, including *Geobacter metallireducens, Desulfovibrio* spp., and *Clostridium.*

Nitrogen assimilation occurs when inorganic nitrogen is used as a nutrient and incorporated into new microbial biomass. Ammonium ion, because it is already reduced, can be directly in- corporated without major energy costs. However, when nitrate is assimilated, it must be reduced with a significant energy expen- diture. In this process nitrite may accumulate as a transient inter- mediate.

Nitrogen fixation can be carried out by aerobic or anaerobic procaryotes and does not occur in eucaryotes. Under aerobic conditions a wide range of free-living microbial genera (*Azotobacter, Azospirillum*) contribute to this process. Under anaerobic conditions the most important free-living nitrogen fixers are members of the genus *Clostridium.* Nitrogen fixation by cyanobacteria such as *Anabaena* and *Oscillatoria* can lead to the enrichment of aquatic environments with nitrogen.. In addition, nitrogen fixation can occur through the activities of bacteria that develop symbiotic associations with plants. These associations include *Rhizobium* and *Bradyrhizobium* with legumes, *Frankia* in association with many woody shrubs, and *Anabaena,* with *Azolla,* a water fern important in rice cultivation.

The nitrogen-fixation process involves a sequence of re- duction steps that require major energy expenditures. Ammo- nia, the product of nitrogen reduction, is immediately incorpo- rated into organic matter as an amine. Reductive processes are extremely sensitive to $O_2$ and must occur under anaerobic con- ditions even in aerobic microorganisms. Protection of the nitrogen-fixing enzyme is achieved by means of a variety of mechanisms, including physical barriers, as occurs with heterocysts in some cyanobacteria, $O_2$ scavenging molecules, and high rates of metabolic activity.

As shown in figure 28.22, microorganisms4 have been isolated that can couple the anaerobic oxidation of $NH^+$ with the reduction of $NO_2^-$, to produce gaseous nitrogen, in what has been termed the anammox process (*an*oxic *amm*onia *ox*idation). This may provide a means by which nitrogen can be removed from sewage plant effluents to decrease nitrogen flow to sensitive freshwater and marine ecosystems. It has been suggested that chemolithotrophic members of the planctomycetes play a role in this process.




**The Basic Nitrogen Cycle.** Flows that occur predominantly under aerobic conditions are noted with open arrows.Anaerobic processes are noted with solid bold arrows. Processes occurring under both aerobic and anaerobic conditions are marked with cross-barred arrows. The anammox reaction of $NO_2^-$ and $NH_4^+$ to yield $N_2$ is shown. Important genera contributing to the nitrogen cycle are given as examples.

**Iron Cycle**

The iron cycle (figure) includes several different genera that carry out iron oxidations, transforming ferrous ion ($Fe^{2+}$) to ferric ion ($Fe^{3+}$). *Thiobacillus ferrooxidans* carries out this process under acidic conditions, *Gallionella* is active under neutral pH conditions, and *Sulfolobus* functions under acidic, thermophilic conditions. Much of the earlier literature suggested that additional genera could oxidize iron, including *Sphaerotilus* and *Leptothrix*. These two genera are still termed "iron bacteria" by many nonmicrobiologists. Confusion about the role of these genera resulted from the occurrence of the chemical oxidation of ferrous ion to ferric ion (forming insoluble iron precipitates) at neutral pH values, where microorganisms also grow on organic substrates. These microorganisms are now classified as chemoheterotrophs.

Recently microbes have been found that oxidize $Fe^{2+}$ using nitrate as an electron acceptor. This process occurs in aquatic sediments with depressed levels of oxygen and may be another route by which large zones of oxidized iron have accumulated in envi- ronments with lower oxygen levels.

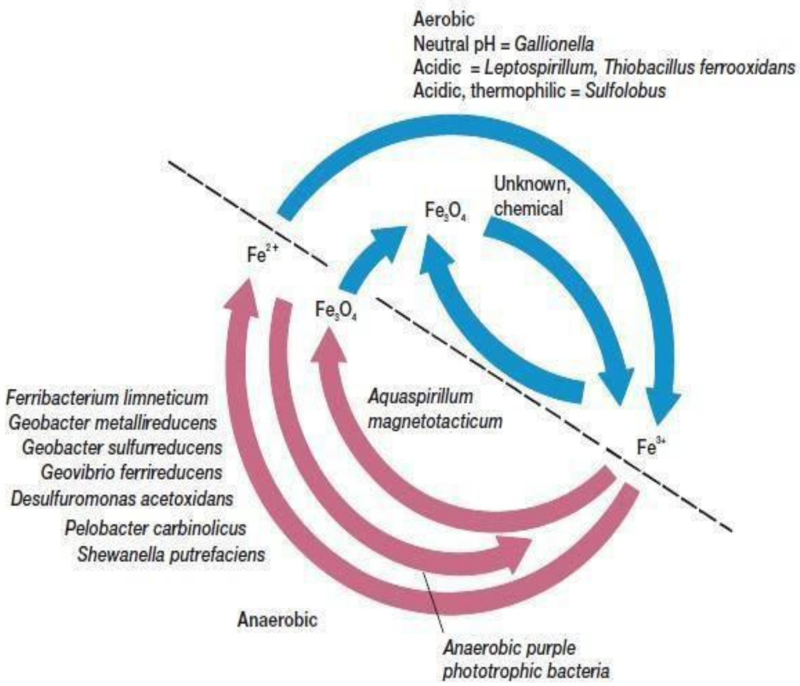
Iron reduction occurs under anaerobic conditions resulting in the accumulation of ferrous ion. Although many microorganisms can reduce small amounts of iron during their metabolism, most iron reduction is carried out by specialized iron-respiring microorganisms such as *Geobacter metallireducens, Geobacter sul-*

*furreducens, Ferribacterium limneticum,* and *Shewanella putre- faciens,* which can obtain energy for growth on organic matter using ferric iron as an oxidant.

In addition to these relatively simple reductions to ferrous ion, some magnetotactic bacteria such as

*Aquaspirillum magnetotacticum* transform extracellular iron to the mixed valence iron oxide mineral magnetite ($Fe_3O_4$) and construct intra- cellular magnetic compasses. Furthermore, dissimilatory iron- reducing bacteria accumulate magnetite as an extracellular product.

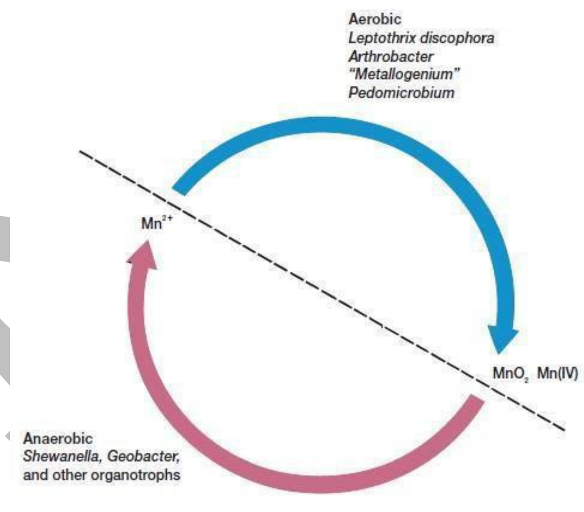
Magnetite has been detected in sediments, where it is present in particles similar to those found in bacteria, indicating a longer- term contribution of bacteria to iron cycling processes. Genes for magnetite synthesis have been cloned into other organisms, cre- ating new magnetically sensitive microorganisms. Magnetotactic bacteria are now described as magneto-aerotactic bacteria, due to their using magnetic fields to migrate to the position in a bog or swamp where the oxygen level is best suited for their func- tioning. In the last decade new microorganisms have been dis- covered that use ferrous ion as an electron donor in anoxygenic photosynthesis. Thus, with production of ferric ion in lighted anaerobic zones by iron-oxidizing bacteria, the stage is set for sub- sequent chemotrophic-based iron reduction, such as by *Geobacter* and *Shewanella*, creating a strictly anaerobic oxidation/reduction cycle for iron.



**The Basic Iron Cycle.** A simplified iron cycle with examples of microorganisms contributing to these oxidation and reduction processes. In addition to ferrous ion ($Fe^{2+}$) oxidation and ferric ion ($Fe^{3+}$) reduction, magnetite ($Fe_3O_4$), a mixed valence iron compound formed by magnetotactic bacteria is important in the iron cycle. Different microbial groups carry out the oxidation of ferrous ion depending on environmental conditions.

## Manganese Cycle

The importance of microorganisms in manganese cycling is be- coming much better appreciated. The manganese cycle (figure) involves the transformation of manganous ion ($Mn^{2+}$) to $MnO_2$ (equivalent to manganic ion [$Mn^{4+}$]), which occurs in hy- drothermal vents, bogs, and as an important part of rock var- nishes. *Leptothrix, Arthrobacter, Pedomicrobium,* and the incom- pletely characterized "*Metallogenium*" are important in $Mn^{2+}$ oxidation. *Shewanella, Geobacter,* and other chemoorganotrophs can carry out the complementary manganese reduction process.




**The Basic Manganese Cycle.** Microorganisms make important contributions to the manganese cycle. Manganous ion (2+**)** is oxidized to manganic oxide (valence equivalent to 4+). Manganous oxide reduction is noted with a maroon arrow. Examples of organisms carrying out these processes are given.

**ENVIRONMENTAL MICROBIOLOGY - 18MBU403**

**Possible Questions – Unit III**

1. Explain in detail about soil profile and soil micro flora.

2. Describe about phosphate immobilisation.

3. Explain in detail about nitrogen cycle.

4. Write a note on phosphorus cycle.

5. Explain in detail about microbes in sulphur cycle.

6. Give an account on microbial degradation of lignin.

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS:II B.Sc MB COURSE NAME: Environmenal Microbiology**
**COURSE CODE: 18MBU403 UNIT-1II BATCH-2018-2021**

| Sno | Question | Option 1 | Option 2 | Option 3 | Option 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | Biogeochemical cycles are also known as | material cycling | gaseous cycle | sedimentary cycling | none of the above | material cycling |
| 2 | Which one is sedimentary cycle? | oxygen cycle | hydrogen cycle | nitrogen cycle | phosphorus cycle | phosphorus cycle |
| 3 | Which gas has greatest effect on global warming? | Ethane | Methane | $CO_2$ | Hydrogen | Methane |
| 4 | The proteinaceous compound are converted to ammonia by | Putrification bacteria | Ammonifiaction bacteria | Nitrification bacteria | Denitrifying bacteria | Ammonifiaction bacteria |
| 5 | Most bacteria require vitamins as | Growth Factors | Sources of energy | Sources of carbon | Sources of electron donars | Growth Factors |
| 6 | Which of these is a trace element for bacteria? | Mg+2 | Na+ | Ca+2 | Mn+2 | Na+ |
| 7 | Nitrites are oxidized to nitrates by a microorganism | Nitrosomonas | Nitrosococcus | Nitrobacter | Azatobacter | Nitrobacter |
| 8 | _________ play a key role in the transformation of rock to soil. | Cyanobacteia | Pectin decomposing bacteria | Nitrifying bacteria | De-nitrifying bacteria | Cyanobacteia |
| 9 | The organisms responsible for the characteristic musty or earth odor of a freshly plowed field is/are | Clostridium | Streptomyces | algae | virus | Streptomyces |
| 10 | Which of the following soil microorganism is involved in the reduction of sulfates to H2S? | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Desulfotomaculum |

| | | | | | | |
|---|---|---|---|---|---|---|
| 11 | Composting is one of the oldest forms of disposal of waste. It is the natural process of decomposition of organic waste that yields manure or compost. One of the following is added to the compost to get better results? | Ants | Bugs | Snakes | worms | worms |
| 12 | Which is not a form of biomass energy? | Incineration of solid waste | Composting to produce methane | Ethanol and methanol production for auto fuel | Photovoltaic production of hydrogen | Photovoltaic production of hydrogen |
| 13 | The transformation of nitrates to gaseous nitrogen is accomplished by microorganisms in a series of biochemical reactions. The process is known as _____________ | nitrification | denitrification | nitrogan fixation | ammonification | Denitrification |
| 14 | Nitrogen fixation refers to the direct conversion of atmospheric nitrogen gas into______ | ammonia | glucose | ATP | Nitrate | ammonia |
| 15 | The diagnostic enzyme for denitrification is__________ | nitrate reductase | nitrate oxidase | nitro oxidoreductase | reductase | nitrate reductase |
| 16 | An example of a symbiotic nitrogen fixer is_________ | Azotobacter | Beijerinckia | Clostridium | Rhizobium | Rhizobium |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 17 | In the process of nitrogen fixation, which of the following microorganism is involved? | Non symbiotic microorganisms only | Symbiotic microorganisms only | Non symbiotic and symbiotic microorganisms only | Symbiosis | Non symbiotic and symbiotic microorganisms only | |
| 18 | The physical structure of soil is improved by the accumulation of ________ | mold mycelium | minerals | water | metals | Mold mycelium | |
| 19 | ________ play a key role in the transformation of rock to soil | Cyanobacteria | Pectin decomposing bacteria | Nitrifying bacteria | De-nitrifying bacteria | Cyanobacteria | |
| 20 | The conversion of molecular nitrogen into ammonia is known as________ | nitrification | denitrification | nitrogen fixation | ammonification | nitrogen fixation | |
| 21 | Some microorganisms have the ability to increase the nitrogen content of soils, are called as | Nitrogen fixation | denitrification | nitrification | ammonification | nitrogen fixation | |
| 22 | Which of the following soil microorganism is involved in the reduction of sulfates to H2S | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Desulfotomaculum | |
| 23 | Most bacteria require vitamins as | Growth Factors | Sources of energy | Sources of carbon | Sources of electron donars | Growth Factors | |
| 24 | Which of these is a trace element for bacteria? | Mg+2 | Na+ | Ca+2 | Mn+2 | Na+ | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 25 | Nitrites are oxidized to nitrates by a microorganism | Nitrosomonas | Nitrosococcus | Nitrobacter | Azatobacter | Nitrobacter |
| 26 | The main product of glycolysis underaerobic conditions is | Pyruvate | Lactate | None of these | Both a and b | Pyruvate |
| 27 | The first Environment Law in India was enacted in__________ | 1950 | 1947 | 1972 | 1982 | 1950 |
| 28 | Bio gas generation is mainly based on the principles of | fermentation | degradation | purification | sedimentation | fermentation |
| 29 | The green house gases, otherwise called radioactively active gases includes___________ | CO2 | O2 | NO2 | NO | CO2 |
| 30 | Which of the following is an organic gas? | Hydrocarbons | Aldehydes | Ketones | Ammonia | Ammonia |
| 31 | What type of energy is derived from heated groundwater? | solar | geothermal | hydroelectric | nuclear | geothermal |
| 32 | ________ is a mixture of 50 - 90%of methane | Natural gas | Air | Water | Bio diesel | Natural gas |
| 33 | Gasification of biomass is one of the means to harvest energy through ________ conversion | hydro-chemical | thermo-chemical | chemical-gaseous | hydro-thermal | thermo-chemical |
| 34 | Gobar gas is obtained from __________ | manure | cow dung | crop residues | fossil | cow dung |

| | | | | | | |
|---|---|---|---|---|---|---|
| 35 | ________ is derived recently from living organisms and their metabolic products. | Biofuel | Biomass | Fossil fuel | Gobar gas | biomass |
| 36 | Biogas is composed of_____ | methane, carbon dioxide, nitrogen and hydrogen | Carbon dioxide, nitrogen and hydrogen | ethane, carbon dioxide, nitrogen and hydrogen | methane, carbon dioxide, nitrogen and sulphur | methane, carbon dioxide, nitrogen and hydrogen |
| 37 | Cows can digest straw because they contain | Cellulose hydrolyzing microorganisms | Protein hydrolyzing bacteria | Lipid hydrolyzing microorganisms | Amino acid degrading bacteria | Cellulose hydrolyzing microorganisms |
| 38 | _________ play a key role in the transformation of rock to soil. | Cyanobacteia | Pectin decomposing bacteria | Nitrifying bacteria | De-nitrifying bacteria | Cyanobacteia |
| 39 | The organisms responsible for the characteristic musty or earth odor of a freshly plowed field is/are | Clostridium | Streptomyces | algae | virus | Streptomyces |
| 40 | Which of the following soil microorganism is involved in the reduction of sulfates to H2S? | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Desulfotomaculum |
| 41 | Cyanobacteria are________ | Photoheterotrophs | Chemotrophs | Prototrophs | Photoautotrophs | Photoautotrophs |
| 42 | Which of the following microorganism use H2S as the electron donor to reduce carbon dioxide? | Chromaticum | Chlorobium | Both a and b | Rhodomicrobium | Both a and b |

| | | | | | | |
|---|---|---|---|---|---|---|
| 43 | What are Blue-Green bacteria called? | Acquaobacteria | Cyanobacteria | Protozoa | None of the above | Cyanobacteria |
| 44 | The Archaea include all of the following except | methanogens | halophiles | thermoacidophiles | cyanobacteria | cyanobacteria |
| 45 | Most abundant pollutant in the atmosphere among hydrocarbons is ________ | methane | propane | butane | benzpyrene | methane |
| 46 | Microaerophilic bacteria are those which require | 21 % oxygen for growth | low levels of oxygen for growth (lesser than O2 present in atmosphere) | oxygen for activation of enzymes | 50 % oxygen for growth | low levels of oxygen for growth (lesser than O2 present in atmosphere) |
| 47 | An organism is completely dependent on atmospheric O2 for growth. This organism is | obligate aerobe | osmotolerant | acidophile | facultative anaerobe | obligate aerobe |
| 48 | Nitrites are oxidized to nitrates by a microorganism | Nitrosomonas | Nitrosococcus | Nitrobacter | Azatobacter | Nitrobacter |
| 49 | The main product of glycolysis underaerobic conditions is | Pyruvate | Lactate | None of these | Both a and b | Pyruvate |
| 50 | ______ normally absorb $CO_2$ from atmosphere | Ocean | River | Lake | Pond | Ocean |
| 51 | Energy resources derived from natural organic materials are called------------ | geothermal energy sources | fossil fuels | biomass | all of these | fossil fuels |
| 52 | A permeable rock that contains hydrocarbon | oil trap | source bed | oil reservoir | none of these | oil reservoir |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | fluids and gasses is called a/an------- | | | | | | |
| 53 | The diagnostic enzyme for denitrification is | nitrate reductase | nitrate oxidase | nitro oxidoreductase | none of these | nitrate reductase | |
| 54 | Which of the following soil microorganism is involved in the reduction of sulfates to H2S? | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Desulfotomaculum | |
| 55 | The global hydrologic cycle supports a net flow of atmospheric water vapour from | land to the oceans | the oceans to land | polar to tropical regions | tropical to polar regions | the oceans to land | |
| 56 | Which of the following is not gaseous type cycle? | carbon cycle | nitrogen cycle | phosphorus cycle | oxygen cycle | phosphorus cycle | |
| 57 | In the phosphorus cycle, phosphorus becomes available by weathering of rock first to | consumers | decomposers | producers | all of these | decomposers | |
| 58 | The main nitrogen reservoir in the biosphere is the | atmosphere | ocean | rocks | organism | atmosphere | |
| 59 | Which of the following atoms most often limits the primary productivity of an ecosystem? | sulphur | phosphorus | nitrogen | Carbon | Carbon | |
| 60 | Among the following biogeochemical cycles which one does not have losses due to respiration? | sulphur | nitrogen | phosphorus | all of above | all of above | |

**Solid waste management**

Solid waste refers here to all non-liquid wastes. In general this does not include excreta, although sometimes nappies and the faeces of young children may be mixed with solid waste. Solid waste can create significant health problems and a very unpleasant living environment if not disposed of safely and appropriately. If not correctly disposed of, waste may provide breeding sites for insect-vectors, pests, snakes and vermin (rats) that increase the likelihood of disease transmission. It may also pollute water sources and the environment.

**Associated risks**

**Disease transmission**

Decomposing organic waste attracts animals, vermin and flies. Flies may play a major role in the transmission of faecal-oral diseases, particularly where domestic waste contains faeces (often those of children). Rodents may increase the transmission of diseases such as leptospirosis and salmonella, and attract snakes to waste heaps.

Solid waste may also provide breeding sites for mosquitoes. Mosquitoes of the *Aedes* genus lay eggs in water stored in discarded items such as tins and drums; these are responsible for the spread of dengue and yellow fevers. Such conditions may also attract mosquitoes of the *Anopheles* genus, which transmit malaria. Mosquitoes of the *Culex* genus breed in stagnant water with high organic content and transmit micro filariases, appropriate conditions are likely to arise where leachate from waste enters pooling water.

In times of famine or food scarcity, members of the affected population may be attracted to waste heaps to scavenge for food; this is likely to increase the risk of gastro-enteritis, dysentery and other illnesses. Pollution

Poor management of the collection and disposal of solid waste may lead to leachate pollution of surface water or groundwater. This may cause significant problems if the waste contains toxic substances, or if nearby water sources are used for water supplies. Where large quantities of dry waste are stored in hot climates this may create a fire hazard. Related hazards include smoke pollution and fire threat to buildings and people.

**Effect on morale**

The effect of living in an unhygienic and untidy environment may lead people to become demoralized and less motivated to improve conditions around them. Waste attracts more waste and leads to less hygienic behavior in general.

**Sources and types of solid waste**

**Sources of solid waste**

In most emergency situations the main sources of solid waste are:

Food stores

Feeding centres

Food distribution points

Slaughter areas

Warehouses

Agency premises

Markets

Domestic areas

**Type and quantity of waste**

The type and quantity of waste generated in emergency situations varies greatly. The main factors affecting these are:

the geographical region (developed or less-developed country or region); socio-cultural practices and material levels among affected population; seasonal variations (affecting types of food available); the stage of emergency (volume and composition of waste may change over time); and the packaging of food rations.

In general, the volume of waste generated is likely to be small and largely degradable where the population is of rural origin and the food rations supplied are unpackaged dry foodstuffs. Displaced urban populations are more likely to generate larger volumes of non-degradable waste, especially where packaged food rations are provided.

**Different categories of solid waste include:**

**Organic waste:** Waste from preparation of food, market places, etc.

**Combustibles:** Paper, wood, dried leaves, packaging for relief items,

**Non-combustibles:** Metal, tin cans, bottles, stones, etc.

**Ashes/dust:** Residue from fires used for cooking

**Bulky waste:** Tree branches, tyres, etc.

**Dead animals:** Carcasses of domestic animals and livestock

**Hazardous waste:** Oil, battery acid, medical waste **Construction waste:** Roofing, rubble, broke concrete, etc.

**Key components of solid waste management**

Solid waste management can be divided into five key components:

**Generation**

Generation of solid waste is the stage at which materials become valueless to the owner and since they have no use for them and require them no longer, they wish to get rid of them. Items which may be valueless to one individual may not necessarily be valueless to another. For example, waste items such as tins and cans may be highly sought after by young children.

**Storage**

Storage is a system for keeping materials after they have been discarded and prior to collection and final disposal. Where on-site disposal systems are implemented, such as where people discard items directly into family pits, storage may not be necessary. In emergency situations, especially in the early stages, it is likely that the affected population will discard domestic waste in poorly defined heaps close to dwelling areas. If this is the case, improved disposal or storage facilities should be provided fairly quickly and these should be located where people are able to use them easily. Improved storage facilities include:

In determining the size, quantity and distribution of storage facilities the number of users, type of waste and maximum walking distance must be considered. The frequency of emptying must also be determined, and it should be ensured that all facilities are reasonably safe from theft or vandalism.

**Collection**

Collection simply refers to how waste is collected for transportation to the final disposal site. Any collection system should be carefully planned to ensure that storage facilities do not become overloaded. Collection intervals and volumes of collected waste must be estimated carefully.

**Transportation**

This is the stage when solid waste is transported to the final disposal site. There are various modes of transport which may be adopted and the chosen method depends upon local availability and the volume of waste to be transported. Types of transportation can be divided into three categories:

**Disposal**

The final stage of solid waste management is safe disposal where associated risks are minimized. There are four main methods for the disposal of solid waste:

Land application: burial or
landfilling Composting
Burning or incineration
Recycling (resource ecovery)

The most common of these is undoubtedly land application, although all four are commonly applied in emergency situations.

**On-site disposal options**

The technology choices outlined below are general guidelines for disposal and storage of waste on-site, these may be adapted for the particular site and situation in question.

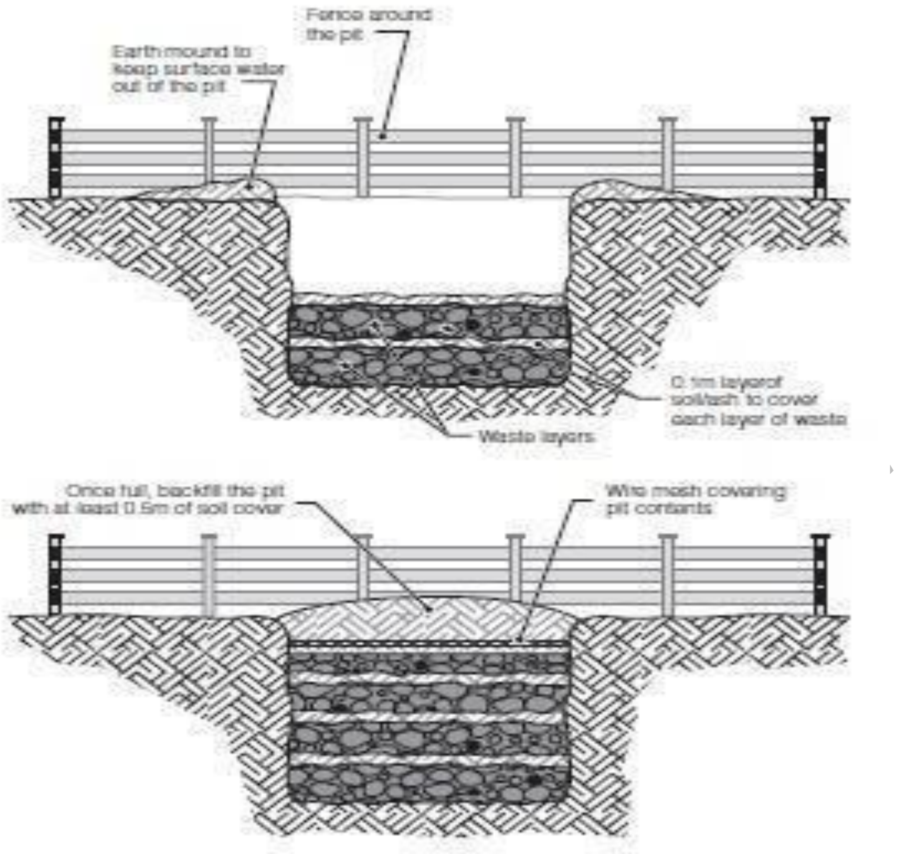
**Communal pit disposal**

Perhaps the simplest solid waste management system is where consumers dispose of waste directly into a communal pit. The size of this pit will depend on the number of people it serves. The long-term recommended objective is six cubic metres per fifty people. The pit should be fenced off to prevent small children falling in and should generally not be more than 100m from the dwellings to be served. Ideally, waste should be covered at least weekly with a thin layer of soil to minimise flies and other pests.

**Advantages:** It is rapid to implement; and requires little operation and maintenance

**Constraints:**

The distance to communal pit may cause indiscriminate disposal; and waste workers required to manage pits.




**Family pit disposal**

Family pits may provide a better long-term option where there is adequate space. These should be fairly shallow (up to 1m deep) and families should be encouraged to regularly cover waste with soil from sweeping or ash from fires used for cooking. This method is best suited where families have large plots and where organic food wastes are the main component of domestic refuse.

**Advantages:** Families are responsible for managing their own waste; no external waste workers are required; and community mobilization can be incorporated into hygiene promo- tion programme.

**Constraints:**

Involves considerable community mobilisation for construction, operation and maintenance of pits; and considerable space is needed.

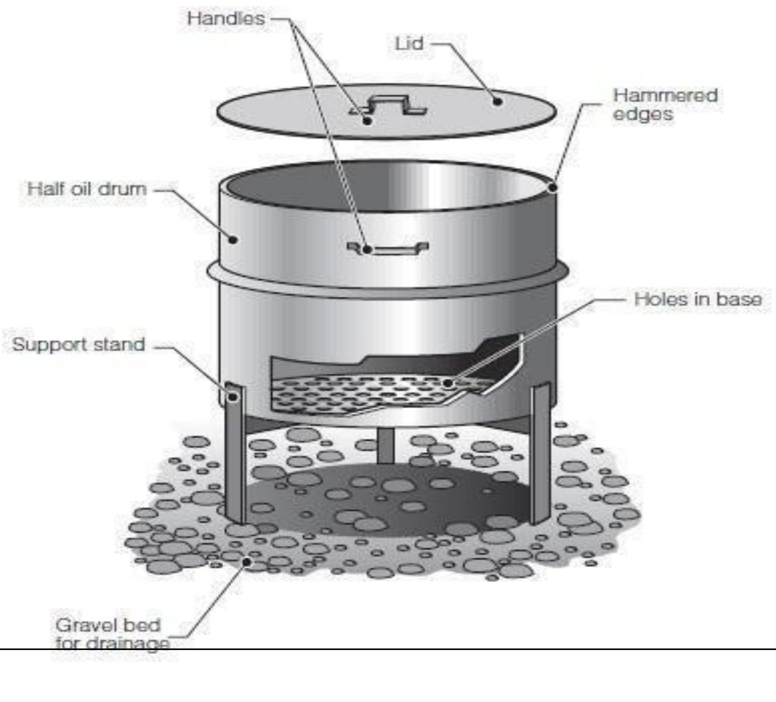
**Communal bins**

Communal bins or containers are designed to collect waste where it will not be dispersed by wind or animals, and where it can easily be removed for transportation and disposal. Plastic containers are generally inappropriate since these may be blown over by the wind, can easily be removed and may be desirable for alternative uses. A popular solution is to provide oil drums cut in half. The bases of these should be perforated to allow liquid to pass out and to prevent their use for other purposes. A lid and handles can be provided if necessary.

In general, a single 100-litre bin should be provided for every fifty people in domestic areas, every one hundred people at feeding centres and every ten market stalls. In general, bins should be emptied daily

**Advantages:** Bins are potentially a highly hygienic and sanitary management method; and final disposal of waste well away from dwelling areas.

**Constraints:** Significant collection, transportation and human resources are required; system takes time to implement; and efficient management is essential.



**Family bins**

Family bins are rarely used in emergency situations since they require an intensive collection and transportation system and the number of containers or bins required is likely to be huge. In the later stages of an emergency, however, community members can be encouraged to make their own refuse baskets or pots and to take responsibility to empty these at communal pits or depots.

**Advantages:** Families are responsible for maintaining collection containers; and potentially a highly sanitary management method.

**Constraints:** In general, the number of bins required is too large; significant collection, transportation and human resources are required; takes time to implement; and efficient management essential.

**Communal disposal without bins**

For some public institutions, such as markets or distribution centres, solid waste management systems without bins can be implemented, whereby users dispose of waste directly onto the ground. This can only work if cleaners are employed to regularly sweep around market stalls, gather waste together and transport it to a designated off-site disposal site. This is likely to be appropriate for vegetable waste but slaughterhouse waste should be disposed of in liquid-tight containers and buried separately.

**Advantages:** System rapid to implement; there is minimal reliance on actions of users; and it may be in line with traditional/usual practice.

**Constraints:** Requires efficient and effective management; and full-time waste workers must be employed.

**Off-site disposal options**

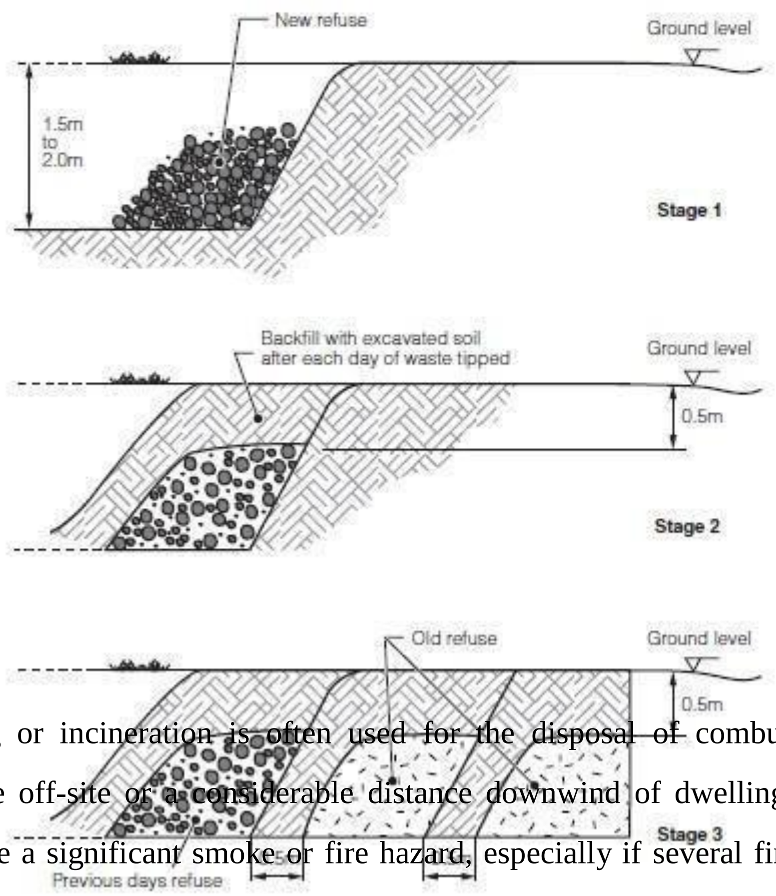
The technology choices outlined below are general options for the final disposal of waste off- site.

**Landfilling**

Once solid waste is transported off-site it is normally taken to a landfill site. Here the waste is placed in a large excavation (pit or trench) in the ground, which is back-filled with excavated soil each day waste is tipped. Ideally, about 0.5m of soil should cover the deposited refuse at the end of each day to prevent animals from digging up the waste and flies from breeding. The location of landfill sites should be decided upon through consultation with the local authorities and the affected population. Sites should preferably be fenced, and at least one kilometre downwind of the nearest dwellings.

**Advantages:** A sanitary disposal method if managed effectively.

**Constraints:** A reasonably large area is required.




**Incineration**

Although burning or incineration is often used for the disposal of combustible waste, this should generally only take place off-site or a considerable distance downwind of dwellings. Burning refuse within dwelling areas may create a significant smoke or fire hazard, especially if several fires are lit simultaneously. Burning may be used to reduce the volume of waste and may be appropriate where there is limited space for burial or landfill. Waste should be ignited within pits and covered with soil once incinerated, in the same manner as landfilling. The same constraints for siting landfill sites should be applied here also.

**Advantages:** Burning reduces volume of combustible waste considerably; and it is appropri- ate in off-site pits to reduce scavenging.

**Constraints:** There can be smoke or fire hazards.

**Composting**

Simple composting of vegetables and other organic waste can be applied in many situations. Where people have their own gardens or vegetable plots, organic waste can be dug into the soil to add humus and fibre. This makes the waste perfectly safe and also assists the growing process. This should be encouraged wherever possible, particularly in the later stages of an emergency programme.

Properly managed composting requires careful monitoring of decomposing waste to control moisture

and chemical levels and promote microbial activity. This is designed to produce compost which is safe to handle and which acts as a good fertiliser. Such systems require considerable knowledge and experience and are best managed centrally. In general, they are unlikely to be appropriate in emergencies.

**Advantages:** Composting is environmentally friendly; and beneficial for crops

**Constraints:** Intensive management and experienced personnel are required for large-scale operations.

**Recycling**

Complex recycling systems are unlikely to be appropriate but the recycling of some waste items may be possible on occasions. Plastic bags, containers, tins and glass will often be automatically recycled since they are likely to be scarce commodities in many situations. In most developing country contexts there exists a strong tradition of recycling leading to lower volumes of waste than in many more developed societies.

**Advantages:** Recycling is environmentally friendly.

**Constraints:** There is limited potential in most emergency situations; and it is expensive to set up.

**Sewage Characteristics**

Characterization of wastes is essential for an effective and economical waste management programme. It helps in the choice of treatment methods deciding the extent of treatment, assessing the beneficial uses of wastes and utilizing the waste purification capacity of natural bodies of water in a planned and controlled manner. While analysis of wastewater in each particular case is advisable, data from the other cities may be utilized during initial stage of planning. Domestic sewage comprises spent water from kitchen, bathroom, lavatory, etc. The factors which contribute to variations in characteristics of the domestic sewage are daily per capita use of water, quality of water supply and the type, condition and extent of sewerage system, and habits of the people. Municipal sewage, which contains both domestic and industrial wastewater, may differ from place to place depending upon the type of industries and industrial establishment. The important characteristics of sewage are discussed here.

**Temperature**

The observations of temperature of sewage are useful in indicating solubility of oxygen, which affects transfer capacity of aeration equipment in aerobic systems, and rate of biological activity. Extremely low temperature affects adversely on the efficiency of biological treatment systems and on efficiency of sedimentation. In general, under Indian conditions the temperature of the raw sewage is observed to be between 15 and 35 0 C at various places in different seasons.

**The pH**

The hydrogen ion concentration expressed as pH, is a valuable parameter in the operation of biological units. The pH of the fresh sewage is slightly more than the water supplied to the community. However,decomposition of organic matter may lower the pH, while the presence of industrial wastewater may produce extreme fluctuations. Generally the pH of raw sewage is in the range 5.5 to 8.0.

**Colour and Odour**

Fresh domestic sewage has a slightly soapy and cloudy appearance depending upon its concentration. As time passes the sewage becomes stale, darkening in colour with a pronounced smell due to microbial activity.

**Solids**

Though sewage generally contains less than 0.5 percent solids, the rest being water, still the nuisance caused by the solids cannot be overlooked, as these solids are highly degradable and there of re-need proper disposal. The sewage solids may be classified into dissolved solids, suspended solids and volatile suspended solids. Knowledge of the volatile or organic fraction of solid, which decomposes, becomes necessary, as this constitutes the load on biological treatment units or oxygen resources of a stream when sewage is disposed off by dilution. The estimation of suspended solids, both organic and inorganic, gives a general picture of the load on sedimentation and grit removal system during sewage treatment. Dissolved inorganic fraction is to be considered when sewage is used for land irrigation or any other reuse is planned.

**Nitrogen and Phosphorus**

The principal nitrogen compounds in domestic sewage are proteins, amines, amino acids, and urea. Ammonia nitrogen in sewage results from the bacterial decomposition of these organic constituents. Nitrogen being an essential component of biological protoplasm, its concentration is important for proper functioning of biological treatment systems and disposal on land. Generally, the domestic sewage contains sufficient nitrogen, to take care of the needs of the biological treatment. For industrial wastewater if sufficient nitrogen is not present it is required to be added externally. Generally nitrogen content in the untreated sewage is observed to be in the range of 20 to 50 mg/L measured as TKN. Phosphorus is contributing to domestic sewage from food residues containing phosphorus and their breakdown products. The use of increased quantities of synthetic detergents adds substantially to the phosphorus content of sewage. Phosphorus is also an essential nutrient for the biological processes. The concentration of phosphorus in domestic sewage is generally adequate to support aerobic biological wastewater treatment. However, it will be matter of concerned when the treated effluent is to be reused. The concentration of PO4 in raw sewage is generally observed in the range of 5 to 10 mg/L.

**Chlorides**

Concentration of chlorides in sewage is greater than the normal chloride content of water supply. The chloride concentration in excess than the water supplied can be used as an index of the strength of the sewage. The daily contribution of chloride averages to about 8 gm per person. Based on an average sewage flow of 150 LPCD, this would result in the chloride content of sewage being 50 mg/L higher than that of the water supplied.

Any abnormal increase should indicate discharge of chloride bearing wastes or saline groundwater infiltration, the latter adding to the sulphates as well, which may lead to excessive generation of hydrogen sulphide.

**Organic Material**

Organic compounds present in sewage are of particular interest for environmental engineering. A large variety of microorganisms (that may be present in the sewage or in the receiving water body) interact with the organic material by using it as an energy or material source. The utilization of the organic material by microorganisms is called metabolism. The conversion of organic material by microorganism to obtain energy is called catabolism and the incorporation of organic material in the cellular material is called anabolism. To describe the metabolism of microorganisms and oxidation of organic material, it is necessary to characterize quantitatively concentration of organic matter in different forms. In view of the enormous variety of organic compounds in sewage it is totally unpractical to determine these individually. Thus a parameter must be used that characterizes a property that all these have in common. In practice two properties of almost all organic compounds can be used: (1) organic compound can be oxidized; and (2) organic compounds contain organic carbon. In environmental engineering there are two standard tests based on the oxidation of organic material: 1) the Biochemical Oxygen Demand (BOD) and 2) the Chemical Oxygen Demand (COD) tests. In both tests, the organic material concentration is measured during the test. The essential differences between the COD and the BOD tests are in the oxidant utilized and the operational conditions imposed during the test such as biochemical oxidation and chemical oxidation. The other method for measuring organic material is the development of the Total Organic Carbon (TOC) test as an alternative to quantify the concentration of the organic material.

**Biochemical Oxygen Demand (BOD)**

The BOD of the sewage is the amount of oxygen required for the biochemical decomposition of biodegradable organic matter under aerobic conditions. The oxygen consumed in the process is related to the amount of decomposable organic matter. The general range of BOD observed for raw sewage is 100 to 400 mg/L. Values in the lower range are being common under average Indian cities.

**Chemical Oxygen Demand (COD)**

The COD gives the measure of the oxygen required for chemical oxidation. It does not differentiate between biological oxidisable and nonoxidisable material. However, the ratio of the COD to BOD does not change significantly for particular waste and hence this test could be used conveniently for interpreting performance efficiencies of the treatment units. In general, the COD of raw sewage at various places is reported to be in the range 200 to 700 mg/L. In COD test, the oxidation of organic matter is essentially complete within two hours, whereas, biochemical oxidation of organic matter takes several weeks. In case of wastewaters with a large range of organic compounds, an extra difficulty in using BOD as a quantitative parameter is that the rate of oxidation of organic compounds depends on the nature and size of its molecules. Smaller molecules are readily available for use by bacteria, but large molecules and colloidal and suspended matters can only be metabolized after preparatory steps of hydrolysis. It is therefore not possible to establish a general relationship between the experimental five-day BOD and the ultimate BOD of a sample, i.e., the oxygen consumption after several weeks. For sewage (with k=0.23 d-1 at 20o C) the BOD5 is 0.68 times of ultimate BOD, and ultimate BOD is 87% of the COD. Hence, the COD /BOD ratio for the sewage is around 1.7.

**Primary, secondary and tertiary sewage treatment**

Contaminated water is involved in the outbreak of most of the major water borne epidemics and hence treatment of waste water before release into environment is becoming more important. With the increasing use of pesticides and other chemicals in our daily lives, this has been given the highest priority in survival of human life. The cases of neglect have been disastrous as seen in the outbreak of cholera and plagues in the past.

Sewage is the main contaminant of waste water. The contaminated water undergoes three stages of treatments. It is initially passed through iron screen which filter out the larger debris. These grit chambers are automatic and the waste water is flown at a constant velocity against the screens which does the filtering.

**Primary treatment**

In this, sedimentation of solid wastes is done by passing the waste water through the tanks. The sludge is then fed to a sludge digester in which further processing is carried out. Alternatively biological treatment is used. The efficiency is higher in terms of unit removal of pollution for the sedimentation process. The primary sludge formed contains almost fifty percent of the suspended solids.

**Secondary treatment**

It involves removal of dissolved and colloidal compounds by the process of oxidation. It is usually done through microorganisms for removal of organic compounds. There are three methods employed depending on the nature of effluent obtained after primary treatment.

**Biofiltration**

It employs the use of intermittent sand filters, contact filters or trickling filters for fine filtration. Filters are costly and employed for smaller volumes of sewage treatment. They also occupy more area. Among these, the trickling filters are the most common and efficient. They are packed bed made of plastic, broken rock, gravel, clinker or slag. The ideal material should be uniformly graded to provide for sufficient voidage.

The effluent can be made to percolate through the bed once again for finer removal of suspended solids. The surface area of the medium, hydraulic loading and temperature of the primary effluent determines the rate of removal of BOD. These filters provide a suitable environment for oxidation of contaminants due to presence of oxidizing microbiota which settles on the filter.

Heterotrophic bacteria and fungi are predominant among the microbes. Autotrophic bacteria occupy the lower layers of the percolation tank. Apart from microbes, macro invertebrates also are present which increases the efficiency due to their grazing nature. It also helps to increase oxygen diffusion.

**Aeration/ Activated sludge process**

These systems treat the waste water by mixing it with a flocculent suspension of microorganisms and aeration of the mixture for long hours sometimes even up to 30 hours depending on the nature of primary effluent. The suspended solids and colloidal matter gets adsorbed on the microbial aggregates. The microbes metabolize these flocs and dissolved nutrients into smaller compounds in a process known as stabilization.

There are three types of activated sludge processes such as conventional, stepped aeration sand contact sterilization systems. The activated sludge is essentially an aquatic system in which the higher links of food webs are absent. The microbial mass has to be maintained by periodic withdrawal of excess sludge from the system. Filter beds are more efficient in oxidizing nitrogen than activated sludge plants.

The microbial community in the sludge is established at two stages one with the untreated waste and another with the purified effluent. Filter beds harbor a succession of communities at different depths. Activated sludge has higher species diversity. They contain more gram negative bacteria and about 200 species of protozoans. The basic process has undergone more revisions and technological improvisations and now it is the most widely used biological waste water treatment process to treat organic and industrial effluents.

**Oxidation ponds**

These are used in warmer climates and makes use of natural water bodies such as lagoons. The waste water is allowed to pass through the lagoon and retained for about 2 to 3 weeks. The organic contaminants undergo bacterial decomposition and carbon dioxide, ammonia and nitrate are released for use by the algal community. Organic sludge settles at the bottom of the pond and methane is finally released. These ponds are prone to harbor pathogens and insects.

**Tertiary treatment**

This is applied to the secondary effluent for maintaining the water quality. The process essentially removes phosphates and nitrates from the system. Rapid sand filters, micro straining and fluidized bed systems are commonly used in tertiary treatment. Activated carbon and sand are typically used. Beds of aquatic macrophytes and reed bed systems are also used in tertiary treatment. The biomass should be harvested frequently to maintain the productivity of the system for efficient functioning.

# ENVIRONMENTAL MICROBIOLOGY - 18MBU403

## Possible Questions – Unit IV

1. Write a note on about solid waste management.
2. Write in detail about tertiary sewage management
3. Briefly explain about types of solid waste.
4. Describe in detail about composting
5. Explain in detail about sanitary landfill.
6. Briefly explain about liquid waste management.
7. Write the methods in solid waste disposal.
8. Discuss in detail about primary sewage treatment
9. Write an account on BOD.
10. Breifly explain oxidation pond and trickling filter

| | **Unit IV** | | | | | |
|---|---|---|---|---|---|---|
| **Sno** | **Question** | **Option 1** | **Option 2** | **Option 3** | **Option 4** | **Answer** |
| 1 | _______ are mixed cultures of naturally occuring beneficial microbes used to degrade contaminants, increase quality of soil etc | Enhanced microorganisms | Efficient microorganisms | Effective microorganisms | Good microorganisms | Effective microorganisms |
| 2 | ________ is a process of bioremediation used to improve quality of underground water | Bioaccumulation | Biostimulation | Bioventing | Biodegradation | Biostimulation and bioleaching |
| 3 | __________ bioremediation involves treating the contaminated material at the site | *Ex situ* | *In situ* | *In vivo* | *In vitro* | *In situ* |
| 4 | ______________ is a method of composting by piling biodegradable waste in long rows | Windrow method | Window method | Landfilling | In-vessel composting | Windrow method |
| 5 | Addition of archaea or bacterial cultures to speed up the rate of degradation of a contaminant | Bioaugumentation | Bioriching | Biobleaching | Biomediation | Bioaugumentation |
| 6 | An engineered device or system that supports a biologically active environment | Incubator | Shake flask | Biofilter | Bioreactor | Bioreactor |
| 7 | Bacteria used in bioleaching | *Vibrio cholerae* | *Salmonella typhi* | *Acidithiobacillus ferrooxidans* | *Lactobacillus caseii* | *Acidithiobacillus ferrooxidans* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8 | Biostimulation is the process of | Organic farming | Enhancing growth conditions of microbes | Metal ore leaching | Isolation & Screening | Enhancing growth conditions of microbes | |
| 9 | Common metal contaminants of sea | Magnesium | Aluminium | Copper | Zinc | copper | |
| 10 | Fungi that have the ability to degrade an extremely diverse range of toxic environmental pollutants | *Aspergillus niger* | *Mucor* | *Fusarium* | *Phanaerochaete chrysosporium* | *Phanaerochaete chrysosporium* | |
| 11 | Intracellular accumulation of environmental pollutants such as heavy metals by living organisms is | Bioriching | Biobleaching | Biomediation | Bioaccumulation | Bioaccumulation | |
| 12 | Oldest form of waste treatment/disposal | Burning | Shredding | Vermicomposting | Landfill | Landfill | |
| 13 | Process of supplying oxygen in situ to microbes in contaminated soil at low flow rates | Bioenhancing | Bioventing | Biomediation | Bioriching | Bioventing | |
| 14 | Technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site | Bioremediation | Disposal | Sewage treatment | Waste removal | Bioremediation | |
| 15 | The use of living microorganism to degrade environment pollutants is called___________ | microremediation | nanoremediation | bioremediation | all of these | bioremediation | |
| 16 | The process of converting environmental polltants into harmless products by naturally occuring microbes is called __________ | bioextraction | microbial extraction | biofiltration | bioleaching | bioleaching | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 17 | Ex situ bioremediation involves the | Degradation of pollutants by microbes directly | Removal of pollutants and collection at a place to faxilitate microbial degradation | Degradation of pollutants by genetically | bioremediation | Removal of pollutants and collection at a place to facilitate microbial degradation | |
| 18 | Which of the following is not an application of bioremediation | Treating oil spills by oil-eating microbes | Sewage treatment by bacteria | Burning of petroleum products floatng on the surface of the sea | Removal of grease by bacteris that digest FOG | Burning of petroleum products floatng on the surface of the sea | |
| 19 | Which of the following is not true with respect to the advantages of bioremediation | Inexpensive | Cost effective | Contaminants are not completely destroyed | Environment friendly and natural process | Contaminants are not completely destroyed | |
| 20 | Bioremediation is the process of removing contaminants or pollutants from the environment to return it to its original state | Chemical | Abiotic | biological | Homeostatic | Biological | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | through the use of __________ mechanisms. | | | | | |
| 21 | The addition of known active microbes to soil or water with the purpose of accelerating microbial processes is called ___________ | Biodegradation | Bioremediation | Bioaccentuation | Bioaugmentation | Biodegradation |
| 22 | Which microorganisms are most commonly used bacteria for bioremediation? | Bacillus | Klebsiella | Staphylococcus | Streptococcus | Bacillus |
| 23 | Advanced treatment is generally used to treat waste water to | remove coarse solids | remove settleable solids | reduce BOD | remove additional objectionable substances | remove additional objectionable substances |
| 24 | The concept of putting microbes to help clean up the environment is called | pasteurization | bioremediation | fermentation | biolistics | bioremediation |
| 25 | Treatment of municipal water supplies is based upon | coagulation, filtration, chlorination | chlorination, filtration, coagulation | filtration, coagulation, chlorination | coagulation, chlorination, filtration | coagulation, filtration, chlorination |
| 26 | which of the following is generally not referred to the sewerage system? | Sanitary sewers | Storm sewers | Combined sewers | Solid sewers | Solid sewers |
| 27 | The magnitude of BOD of wastewater is related to | bacterial count | amount of organic material | amount of inorganic material | all of the above | amount of organic material |
| 28 | *Chlorella pyrenoidosa* is usually found in | Activated sludge process | Sludge compost | Trickling filter | Oxidation pond | Activated sludge process |
| 29 | Schmutzdecke is a hypogeal biological layer formed on surface of slow sand filter by | Fungi | Bacteria | Protozoa | Algae | Bacteria |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 30 | Sludge conditioning is accomplished by which of the following | Thickening | Elutriation | Chemical conditioning | Diluting with water | Thickening | |
| 31 | A dense bacterial population caught in a tangled web of fibers sticking to a surface describes | biodisc | coagulation | the membrane filter technique | a biofilm | a biofilm | |
| 32 | The filtering medium of the tank becomes coated with a microbial flora, the ___________ film | Biofilm | Zooglocal film | Neustonic | Algal bloom | Zooglocal film | |
| 33 | The most commonly efficient substrate used as a carbon source indenitrification during sewage treatement is | Methanol | Oxygen | Glucose | Sucrose | Methanol | |
| 34 | The optimum rate of relative humidity for the survival of the most microorganisms is | 40-80% | 60-80% | 50-80% | 30-80% | 40-80% | |
| 35 | Which of the following is not an aerobic process? | Activated sludge process | Sludge digestion | Trickling filter | Oxidation pond | Trickling filter | |
| 36 | Zoogloeal film formed in the trickling filter consists of | Bacteria | Algae | Protozoa | Algal bloom | Bacteria | |
| 37 | Biogas production is | a temperature independent process | a temperature-dependent process | an oxygen dependent process | none of the above | a temperature-dependent process | |
| 38 | Which of the following method is used for removal of suspended materials from waste water | Filration | Purification | sedimentation | settlement | Sedimentation | |
| 39 | Which of the following promotes the biological transformation of dissolved | Primary | Secondary | Tertiary | Quaternary | Secondary | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | organic matter to microbial biomass and carbondioxide | | | | | |
| 40 | Inorganic nutrients are removed by biological means refer as which of the following treatment process | Primary | Secondary | Tertiary | Quaternary | Tertiary |
| 41 | In industrial processing plants, which of the following is the principle factor of treating waste water | Removal of microbes | Removal of organics | Removal of solids | Removal of liquids | Removal of organics |
| 42 | Along with inorganic and organic nutrients which of the following compounds are removed through tertiary treatment process | Heavy and trace metals | Lignocellulosic | Suspended matters | Floating materials | Heavy and trace metals |
| 43 | Algal bloom results in __________ | Global warming | Salination | Eutrophication | Biomagnification | Eutrophication |
| 44 | Copper is used in water treatement as a | Disinfectant | Indicator | Coagulant | Flocculants | Disinfectant |
| 45 | Formation of _______ is crucial step in anaerobic digestion | Hydrogen | Carbondioxide | Water | Acetate | Acetate |
| 46 | Most abundant pollutant in the atmosphere among hydrocarbons is _________ | methane | propane | butane | benzpyrene | methane |
| 47 | Which of the following bacterium is called as the superbug that could clean up oil spills | Bacillus subtilis | Pseudomonas putida | Pseudomonas denitrificans | Bacillus denitrificans | Pseudomonas putida |
| 48 | Which of the following is not employed as an oxidation method? | Trickling filters | Oxidation ponds | Contact aerators | All of these | All of these |

| | | | | | | |
|---|---|---|---|---|---|---|
| 49 | What is an anaerobic digester? | Digests food | Microbe that eats hazardous waste | Method to convert agricultural waste into a biogas | All of the above | Method to convert agricultural waste into a biogas |
| 50 | The biogas production process takes place at the temperature | lesser than 25°C | 25-40°C | 45-60°C | all of these | all of these |
| 51 | Activated sludge contains large number of | bacteria | yeasts and molds | protozoa | all of these | all of these |
| 52 | Trickling filter comes under | primary treatment | secondary treatment | tertiary treatment | none of these | secondary treatment |
| 53 | Removal of solids is generally considered as a | Primary treatment | Secondary treatment | Tertiary treatment | quarternary treatment | Primary treatment |
| 54 | Microaerophilic bacteria are those which require | 21 % oxygen for growth | low levels of oxygen for growth (lesser than O2 present in atmosphere) | oxygen for activation of enzymes | 50 % oxygen for growth | low levels of oxygen for growth (lesser than O2 present in atmosphere) |
| 55 | An organism is completely dependent on atmospheric O2 for growth. This organism is | obligate aerobe | osmotolerant | acidophile | facultative anaerobe | obligate aerobe |
| 56 | Biomass | provides about 50% of its energy | consists largely of wood, animal, and human waste | is unlikely to be a major source of energy globally | offers the consumer high quality energy with low environmental impact | consists largely of wood, animal, and human waste |
| 57 | The acetate-utilizing methanogens are responsible for | 20% of methane produced in a biogas reactor | 50% of methane produced in a biogas reactor | 70% of methane produced in a biogas reactor | 85% of methane produced in a biogas reactor | 70% of methane produced in a biogas reactor |

Introduction

Pesticides can be used to control or to manage pest populations at a tolerable level. The suffix "-cide" literally means "kill", therefore, the term pesticide refers to a chemical substance that kills pests. It is incorrect to assume that the term pesticide refers only to insecticides. Pesticides include many different types of products with different functions or target (Table 1). The pesticide designation is formed by combining the name of the pest (e.g., insect or mite) with the suffix "-cide".

Pesticides could be classified according to their toxicity, chemical group, environmental persistence, target organism, or other features. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 persistent organic chemicals are pesticides. Classes of organic pesticides (consisting of organic molecules) include organochlorine, organophosphate, organometallic, pyrethroids, and carbamates among others.

Most pesticides cause adverse effects when reaching organisms. The intensity of the toxic effect varies with time, dose, organism characteristics, environmental presence or pesticide characteristics. Their presence in environment determines the dose and time at which an organism is exposed and could represent a hazard for worldwide life due to their mobility. Hence, the persistence in the environment leads to a risk for life: the more persistent a pesticide is, the worse its environmental impact.

Pesticide persistence in environment is caused by either their physico-chemical properties or the lack of organisms able to degrade them. Light, heat or humidity could lead to loss of some pesticides by either volatilization or degradation. Contrastingly, degradation caused by organisms (biodegradation) could help decreasing considerably the pesticides persistence in environment. This information could be used to improve elimination of the undesirable effects of pollutants by using organisms; such an approach has been called bioremedation. The ability of organisms to bioremediate pesticides is mainly based on their biodegradation activity. Though bioremediation has been firstly achieved using microorganisms (bacteria or fungi), other organisms like plants or algae can be used. The aim of the present paper is to review the metabolic features which make organisms useful for bioremediation.

**Overview**

At this point, it is worth to mention that there is no convention on some words used in biodegradation. Here, we propose some words to improve communication and understanding bioremediation strategies. Albeit discussion of proper words is beyond aim of the present paper, we believe that before continuing is important to set up some concepts.

"Bioremediation" refers to any strategy used to eliminate undesirable effects of pollutants from environment. It would be desirable to eliminate pollutants but this is not always possible; though, some organisms could confine or immobilize them. For instance, organisms can accumulate contaminants, and reduce their presence and their environmental effect, but do not eliminate them from the environment. Such strategy, which is actually used should be included into the "bioremediation" concept. Those organisms able to bioremediate would be called bioremediators.

| Pesticide | Target |
|---|---|
| Algicides | Algae |
| Avicides | Birds |
| Bactericides | Bacteria |
| Fungicides | Fungi |
| Insecticides | Insects |
| Miticides or Acaricides | Mites |
| Molluscicides | Snails |
| Nematicides | Nematodes |
| Rodenticides | Rodents |
| [redacted] | |
| Virucides | Viruses |
| [redacted] | |

Table 1. Classification of pesticides according to their target.

Traditionally, bioremediation has been achieved by using microorganisms. Nevertheless, The fact that in past decades, several reports on bioremediation using plants, fungi, algae or enzymes (obtained from organisms) has broadened the scope of bioremediation. Words like phytoremediation or rhizoremediation have been used, and perhaps it would be necessary to name properly each bioremediation strategy regarding the organism used (Table 2).

**Bioremediator organism Strategy**

| | |
|---|---|
| Microorganism | Microbioremediation or Bioremediation |
| Bacteria | Bacterial bioremediation |
| Fungi | Mycoremediation |
| Plants | Phytoremediation |
| Rhizosphere | Rhizoremediation |
| Algae | Phycoremediation |
| Biomolecules derived from organisms | Derivative bioremediation |

**Table 2.** Classification of bioremediation strategies according to the organism involved.

The concepts of biodegradation and biotransformation overlap extensively, so that, they are synonymous in appearance. Biodegradation involves the biological reactions that modify the chemical structure of the compound, so, this implies a decrease in toxicity. In contrast, biotransformation reduces the pollutant concentration by either modification or translocation. Thus, biotransformation could end decreasing or increasing the undesirable effects. Their difference is clear in the case of pollutants translocation when biodegradation is not occurring but biotransformation does. Biotransformation concept has been developed for biological detoxification systems and is a key concept in bioremediation strategies because they both are intended to eliminate undesirable effects of pollutants to organisms. Along the text, the word "Biodegrader" will be used for the organism able to biodegrade a certain compound. "Mineralization" refers to biodegradation leading to compounds like CO2 or NH3 which could be biologically assimilated.

In the earliest works on bioremediation, the practical purpose was to find or to isolate biodegrader microorganisms or consortia. In an admirable work, Alexander reviewed several biodegrader consortia found in polluted environmental matrixes (soil, sediment or water). Among those tolerant or adapted microorganisms, there might be some proper bioremediators. A plausible explanation for this phenomenon might be that pesticides have exerted evolutionary pressure, so that, only organisms able to tolerate those doses of pesticides will survive. Even though not every tolerant organism is a biodegrader, every biodegrader should be tolerant. Thus, the evolutionary pressure exerted by the pollutant would have selected some tolerant bioremediators. In keeping with this, traditionally, bioremediation studies measured only final concentration of pollutants, but little or no attention to biochemical mechanisms responsible for biodegradation was given. Further research on factors affecting biodegradation process is required to improve selection of bioremediators and application of bioremediation technologies.

**Factors affecting biodegradation process**

Some metabolic features related to biodegradation efficiency have been investigated for microorganisms. Any factor which can alter growth or metabolism, would also affect biodegradation. Hence, physicochemical characteristics of the environmental matrix, such as temperature, pH, water potential, oxygen and substrate availability, would influence the biodegradation efficiency (Figure 1). Two more factors are worth to mention: co-metabolism and consortia condition. Some biodegraders need other substrates to degrade pollutants (8). This phenomenon is called co-metabolism and is especially required for organochlorine compounds. In contrast, it has been shown that the presence of other carbon sources decreases organophosphate biodegradation.

When pesticide degradation occurs, it usually involves more than one microorganism, i.e. each microorganism contributes to biodegradation reactions on pesticides, but no example of mineralization by a single strain has been described. It seems that the presence of different microorganisms is essential for an adequate biodegradation. Reported microbiodegraders belong to basidiomycetes or to bacterial classes: gamma-proteobacteria (v.gr.: Pseudomonas, Aerobacter, Acinetobacter, Moraxella, Plesiomonas), beta-proteobacteria (v.gr.: Burkholderia, Neisseria), alpha-proteobacteria (v.gr.: Sphingomonas), actinobacteria (Micrococcus) and flavobacteria (Flavobacterium).

Pollutants might undergo biodegradation reactions like de-chlorination, cleavage, oxidation, reduction by different enzymes. Since biodegradation ability is based on enzymes which are promiscuous and have evolved to detoxifying enzymes, the shorter the duplication time of organism, the more adequate the organism is and the easier to obtain biodegraders. Thus, bacteria with duplication time around minutes are likeable to respond to natural or artificial pollutant-induced evolutionary pressure; this response consists in selecting biotransformation enzymes able to degrade them. These promiscuous enzymes are present in organisms even before the exertion of the evolutionary pressure, which could have induced genetic recombination or mutation leading to enzymes with better biodegradation ability. Copley has excellently reviewed the evolution of metabolic pathways and those factors affecting the efficiency of pollutant biodegradation.

Though bacteria have been proved to be good biodegraders and bioremediators, some fungi, plants and algae could biodegrade pesticides too. Knowing the metabolism of those biodegrader species or strain improves the selection of bioremediation strategy for each site either by biostimulating the indigenous biodegraders (biostimulation) or adding exogenous to the site (bioaugmentation). Moreover, thanks to molecular biology, the metabolic biodegradation ability could be transferred from a biodegrader to another organism, thus improving its degrading capabilities. For instance, using genetic engineering, a whole mineralization pathway for paraoxon –the oxon metabolite of the organophosphate pesticide parathion- was built in a single strain of Pseudomona putida. Taking all this into account, it is clear that biodegradation enzymes play a key role in bioremediation processes and their knowledge could help in designing or choosing the most adequate strategy.

Biotransformation enzymes have been traditionally classified according to the phase they participate. There are three phases of biotransformation. Phase I consists of those enzymes catalyzing reactions which modify pollutant functional groups. In phase II, those enzymes catalyzing transfer reaction of whole groups or biomolecules to pollutants are classified. Phase III includes translocation processes rendering pollutants or their metabolites non bioavailable. For bioremediation purposes, biotransformation enzymes mainly belong to four biochemical types: oxidoreductases, hydrolases, transferases and translocases (or pumps). Among oxidoreductases, the most frequent are monooxygenases (like cytochrome P450), dioxygenases, peroxidases and oxidases. Hydrolases like A-esterases are involved in biodegradation pathways. There are many types of transferases, and they are classified according to the group they conjugate to the xenobiotic: methyl-transferases, acetyl- transferases, glutathione S-transferases among others. For bioremediation purposes, only a couple of translocases have been identified and characterized: boths are pumps that translocate herbicides or glutathione-conjugates to vacuoles.

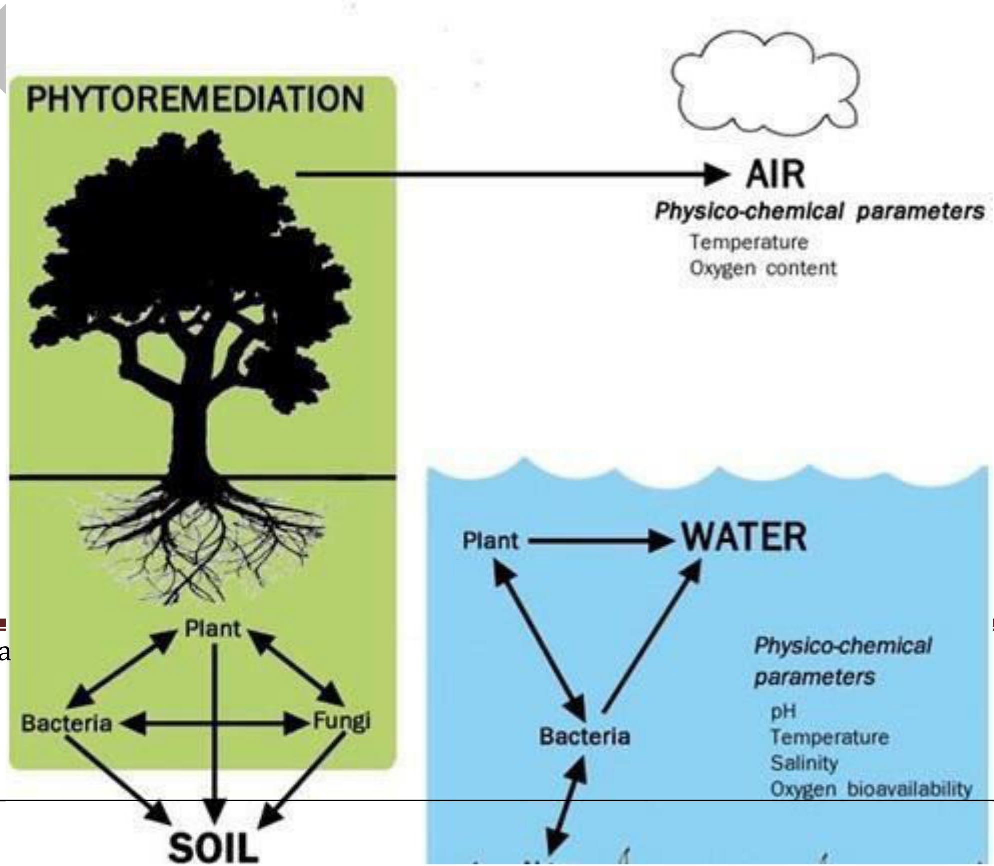


Figure 1. Factors affecting biodegradation and bioremediation in soil, water or air.

The biotransformation of every pollutant could be catalyzed by different enzymes depending on organism. There is no a sequence of reaction pre-determined and is independent of the classification described above. Detoxifying enzymes are promiscous and have different affinities and velocities. Their protein nature makes them susceptible to different factors like heat, pH or substrate availability. In general, biotransformation enzymes for bioremediation are present in bacteria, fungi, plants and animals. In the next section, main enzymes from bacteria, fungi and plants involved in organic pesticide degradation are briefly described. Afterwards, some examples of bacterial, plant, fungi or algae bioremediators are reviewed.

Cytochrome P450 (CYP): This consists of a superfamily of heme monooxygenases. They can catalyze reactions of oxidation, reduction or oxidative breakdown of xenobiotics (Figure 2). It seems that they are evolutively conserved since genomes from virus, bacteria, algae, plant, fungi and animals have isoforms of CYP codified. In eukaryotic organisms, CYP is found in smooth endoplasmic reticulum, and can biotransform a wide range of pollutants. A review about the biology of CYP can be found elsewhere. CYP catalyzes biodegradation of aromatic or alyciclic compounds and can activate toxics, i.e., CYP action on biomolecules might make them toxic or increase their toxicity.

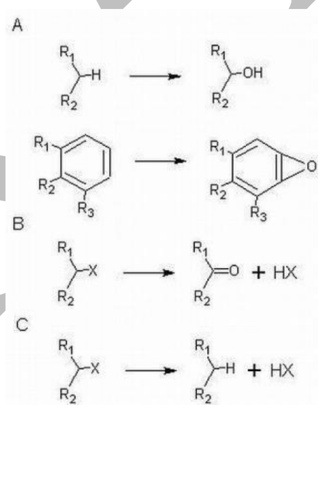


**Figure 2.** Scheme of reactions catalyzed by CYP: A) oxidation (monooxygenation), B) oxidative and C) reductive dehalogenation.

**A-esterases**:

Esterases can be classified according to their interaction with organophosphates. A-esterases can catalyze the hydrolysis of organophosphate or carbamate pesticides (Figure 3), B-esterases are inhibited by organophosphates and C- esterases show no interaction with organophosphates. A-esterases include several enzymes like monophosphatases, phosphodiesterases or phosphotriesterases. They frequently use calcium and have been found in bacteria, fungi and animals. Human paraoxonase is an A-esterase and is involved in susceptibility to organophosphate pesticides; a review on human PON1 could be found elsewhere.

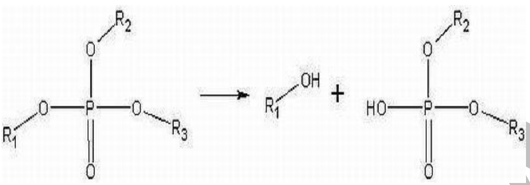


Figure 3. Scheme of reactions catalyzed by A-esterases.

**Peroxidases and oxidases:**

They include some families of enzymes catalyzing redox reactions (Figure 4). Although they are produced by bacteria, fungi, plants and animals, reports on pesticide biodegradation exist for fungi. Peroxidases participate in cell response to oxidative damage and most of them are metalloproteins. They are extemely sensitive to the presence of azide, and inhibitor of metalloenzymes, with the exception of lignine peroxidises from fungi. It is known that ligninolytic fungi secrete peroxidases and oxidases to degrade lignine. These enzymes are highly promiscuous.

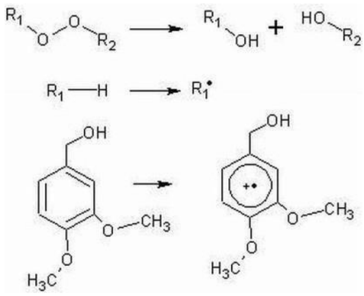


Figure 4. Scheme of reactions catalyzed by peroxidases.

**Transferases:**

Among all known transferases, Glutathione S-transferase (GST) is the mainly involved in biodegradation for bioremediation purposes. GST includes a superfamily of enzymes that have been found in bacteria, fungi, algae, plants and animals. Even though they catalyze transference of glutathione to electrophillic pesticides, they can also show hydrolytic and peroxidase activities. Interestingly, GST can also catalyze the de- halogenation of rings (Figure 5).

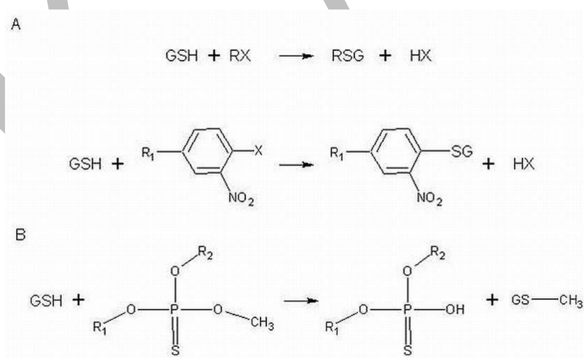


Figure 5. Scheme of reactions catalyzed by GST: A) dehalogenation, B) O-dealkylation.

**Translocases:**

Translocation of molecules from a cell compartment to another is catalyzed by pumps named translocases. Some translocases are involved in the bacterial resistance to drugs, but this activity seems to lack relevance for bioremediation. Although it does not constitute a biodegradation itself, translocation is perhaps the only step of phase III biotransformation. In plants, translocation is part of secondary metabolism and herbicide- tolerance; interestingly, it has been suggested that a previous glutathionation is required for translocation to vacuoles .

**Bacterial bioremediators**

Bacteria have been used extensively for bioremediation purposes. These studies have focused on the employment of bacteria, consortia or on the search for biotransformation enzymes. The fast growth, easy handling and low cost make them suitable for bioremediation. Unfortunately, there are some disadvantages such as the disposal of bacterial biomass, pathogenicity, bioactivation, among others. Bacteria can be found in soil, water or even in particles dispersed in air. Unfortunately, only a small fraction of bacteria (<10% from soil) can be cultured in laboratory conditions. Because of this, the number of studies about pesticide biodegradation mechanisms is less than those about biodegraders isolation, and then, little information on biochemical mechanisms or enzymes is available. For organochlorine pesticides, only few biodegration enzymes and genes have been described.

Bacterial biodegradation could take place in anaerobic or aerobic conditions. Although different enzymes participate in each condition, it seems that both, aerobic and anaerobic degradation should happen if a mineralization is expected to occur. It seems that anaerobic metabolism is more adequate for dechlorination and aerobic metabolism produces a cleavage in aromatic or aliphatic cyclic metabolites. The higher persistence of organochlorine in aerobic conditions compared to anaerobic might be caused by the absence of enzymes or more likely by the oxidative damage following organochlorine metabolism. The removal of heteroatoms (like halogens) or heteroatom-containing groups are frequently among the first steps in biodegradation. These steps are catalyzed by monooxygenases, dioxygenases or peroxidases , which in aerobic conditions could generate large quantities of free radicals. Thus, anaerobic conditions are more adequate for biodegradation of organochlorine pesticides, while aerobic are better for biodegrading hydrocarbon metabolites from pesticides. In spite of such requirements, some examples of organochlorine pesticides bioremediation could be accomplished in situ.

Baczynski and co-workers demonstrated that anaerobic biodegradation of dichlorodiphenyltrichloroetano (DDT), metoxychlor and gamma-hexachlorociclohexane (gamma-HCH), is affected by temperature and the ratio of desorbed pesticide. Moreover, only on chlorine atom could be cleaved from DDT in those conditions. This is in agreement with that reported by Alexander who pointed out that biodegradation could produce molecules with at least one chlorine atom. Bacteria related to Pseudomonas, Neisseria, Moraxella and Acinetobacter able to degrade almost completely DDT were isolated from Yaqui valley in Sonora, Mexico. However, no information on biodegradation mechanism was compiled out.

Anabaena (a cyanobacterium), Pseudomonas spinosa, Pseudomonas aeruginosa and Burkholderia were shown to be good biodegraders of endosulfan. The biodegrader KS-2P strain of Pseudomona was isolated from endosulfan polluted soil by repetitive enrichment in cultures. This strain could reduce the endosulfan concentration in days in a dose-dependent manner. As far as we know, no mineralization of endosulfan has been observed. Microorganisms from the Pseudomonas, Bacillus, Trichoderma, Aerobacter, Muchor, Micrococcus and Burkholderia genera have been shown to biodegrade dieldrin and endrin.

Even when HCH is considered as a persistent organic pollutant, it has been demonstrated that it could be bioremediated in situ. Murthy and Manonmani identified a HCH- biodegrader consortium which contained species from Pseudomonas, Burkholderia, Flavobacterium and Vibrio genera. The biodegradation was achieved within hours. An excellent review by Phillips and co-workers describes and enlists several HCH biodegraders. Interestingly, they could be grouped in two bacteria (Sphingomonas and Pseudomonas) and one white rot fungi (Phanerochaete chrysosporium). HCH mineralization seems to need aerobic and anaerobic conditions like those provided by particles, i.e. in one hand, oxygen could be bioavailable in soil, on the other, soil particles may present niches for anaerobic metabolism. This could explain also why bacteria grown on coffee beans exhibit better biodegradation than those in medium alone. Genes encoding enzymes able to degrade gamma-HCH have been named lin , but further research on biochemical characterization is needed. Comparing biodegradation times for HCH, DDT and endosulfan, differences are observed. Listed in an increasing order of needed time for biodegradation: HCH<DDT<endosulfan. Evidently, this time varies according to the consortium or strains used.

It has been shown that some bacteria could degrade parathion and fenitothrion by using A-esterases. From soil, Singh et al. have isolated a strain related to Enterobacter which can mineralize chlorpyrifos, parathion, diazinon, coumaphos and isazofos. Similarly, it has been found that a bacterial biodegrader related to Serratia can degrade diazinon. The A-esterase, can be encoded on genome or plasmid. A gene from the genome of a strain related to Plesiomonas which can hydrolize methylparathion was cloned to Escherichia coli. In contrast, the ability to degrade fenitothrion by a Burkholderia strain was found to be encoded on plasmids. Unfortunately, the presence of other carbon or phosphorous sources reduces the efficiency of organophosphate biodegradation. This limits severely the application of these biodegraders on bioremediation. Further research about parameters influencing biodegradation efficiency is needed to improve their usefullness for bioremediation.

**Role of microorganisms in biodegradation of pollutants**

Biodegradation is described associated with environmental bioremediation. Therefore, biodegradation is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used and reused by other organisms. In the microbiological sense, "biodegradation" means that the decaying of all organic materials is carried out by a huge assortment of life forms comprising mainly bacteria, yeast and fungi, and possibly other organisms. Bioremediation and biotransformation methods endeavour to harness the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), radionuclides and metals .

**Some biodegradable pollutants**

In the last few decades, highly toxic organic compounds have been synthesized and released into the environment for direct or indirect application over a long period of time. Fuels, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides and dyes are some of these types of compounds. Some other synthetic chemicals like radionuclides and metals are extremely resistant to biodegradation by native flora compared with the naturally occurring organic compounds that are readily degraded upon introduction into the environment.

**Hydrocarbons:** are organic compounds whose structures consist of hydrogen and carbon. Hydrocarbons can be seen as linear linked, branched or cyclic molecules. They are observed as aromatic or aliphatic hydrocarbons. The first one has benzene ($C6H6$) in its structure, while the aliphatic one is seen in three forms: alkanes, alkenes and alkynes .

**Polycyclic aromatic hydrocarbons (PAHs):** are important pollutants class of hydrophobic organic contaminants (HOCs) widely found in air, soil and sediments. The major source of PAH pollution is industrial production . They have been studied with increasing interest for more than twenty years because of more findings about their toxicity, environmental persistence and prevalence . PAHs can sorb to organic-rich soils and sediments, accumulate in fish and other aquatic organisms, and may be transferred to humans through seafood consumption . The biodegradation of PAHs can be considered on one hand to be part of the normal processes of the carbon cycle, and on the other as the removal of man-made pollutants from the environment. The use of microorganisms for bioremediation of PAH-contaminated environments seems to be an attractive technology for restoration of polluted sites.

**Polychlorinated biphenyls (PCBs):** are mixtures of synthetic organic chemicals. Due to their non- flammability, chemical stability, high boiling point, and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment; as plasticizers in paints, plastics, and rubber products; in pigments, dyes, and carbonless copy paper; and many other industrial applications. Consequently, PCBs are toxic compounds that could act as endocrine disrupters and cause cancer. Therefore, environmental pollution with PCBs is of increasing concern

**Pesticides:** are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides which are rapidly degraded are called nonpersis- tent while those which resist degradation are termed persistent. The most common type of degradation is carried out in the soil by microorganisms, especially fungi and bacteria that use pesticides as food source.

**Dyes:** are widely used in the textile, rubber product, paper, printing, color photography, pharmaceuticals, cosetics and many other industries. Azo dyes, which are aromatic compounds with one or more (–N=N–) groups, are the most important and largest class of synthetic dyes used in commercial applications. These dyes are poorly biodegrabale because of their structures and treatment of wastewater containing dyes usually involves physical and / or chemical methods such as adsorption, coagulation-flocculation, oxidation, filtration and electrochemical methods . The success of a biological process for color removal from a given effluent depends in part on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures.

**Radionuclides:** a radionuclide is an atom with an unstable nucleus, characterized by excess energy available to be imparted either to a newly created radiation particle within the nucleus or via internal conversion. During this process, the radionuclide is said to undergo radioactive decay, resulting in the emission of gamma ray(s) and/or subatomic particles such as alpha or beta particles.

**Heavy metals:** unlike organic contaminants, the metals cannot be destroyed, but must either be converted to a stable form or removed. Bioremediation of metals is achieved through biotransformation. Mechanisms by which microorganisms act on heavy metals include biosorption (metal sorption to cell surface by physicochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes), intracellular accumulation, and enzyme-catalyzed transformation (redox reactions) . The major microbial processes that influence the bioremediation of metals are summarized in Figure 2

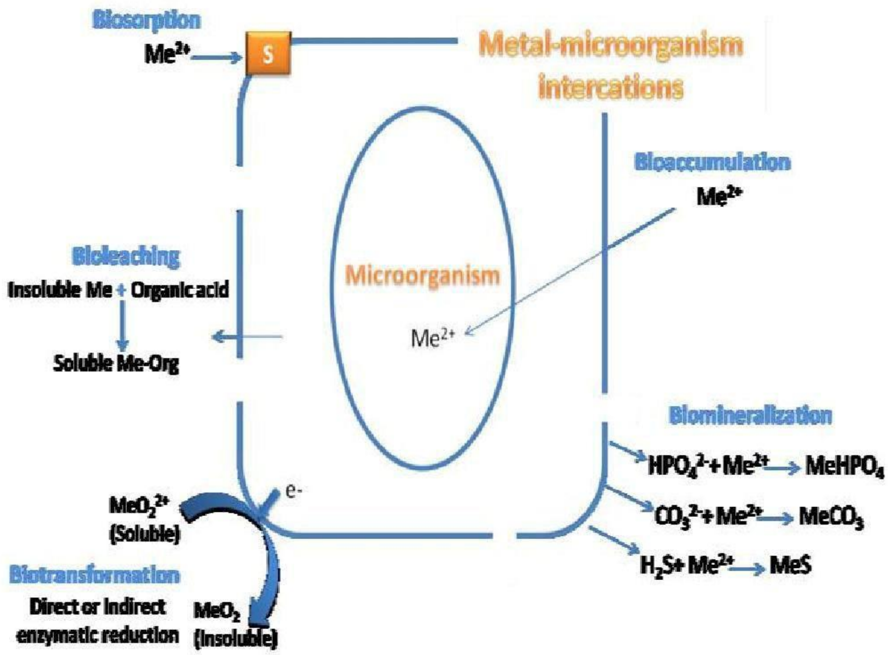



Figure 2. Microbial processes used in bioremediation

**Bacterial degradation**

There are many reports on the degradation of environmental pollutants by different bacteria. Several bacteria are even known to feed exclusively on hydrocarbons . Bacteria with the ability to degrade hydrocarbons are named hydrocarbon-degrading bacteria. Biodegradation of hydrocarbons can occur under aerobic and anaerobic conditions, it is the case for the nitrate reducing bacterial strains Pseudomonas sp. and Brevibacillus sp. isolated from petroleum contaminated soil. However, data presented by Wiedemeier et al. suggest that the anaerobic biodegradation may be much more important. 25 genera of hydrocarbon degrading

bacteria were isolated from marine environment. Furthermore, among 80 bacterial strains Isolated belonged to 10 genus as follows: Bacillus, Corynebacte- rium, Staphylococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia, Klebsiella and Enterobacter, Bacillus was the best hydrocarbon degrading bacteria.

Bacterial strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated, mainly from soil. These are usually gram negative bacteria, most of them belong to the genus Pseudomonas. The biodegradative pathways have also been reported in bacteria from the genera Mycobacterium, Corynebacterium, Aeromonas, Rhodococcus and Bacillus.

Although many bacteria are able to metabolize organic pollutants, a single bacterium does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil. Mixed microbial communities have the most powerful biodegradative potential because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas.

Both, anaerobic and aerobic bacteria are capable of biotransforming PCBs. Higher chlorinated PCBs are subjected to reductive dehalogenation by anaerobic microorganisms. Lower chlorinated biphenyls are oxidized by aerobic bacteria. Research on aerobic bacteria isolated so far has mainly focused on Gram-negative strains belonging to the genera Pseudomonas, Burkholderia, Ralstonia, Achromobacter, Sphingomonas and Comamonas. However, several reports about PCB-degrading activity and characterization of the genes that are involved in PCB degradation indicated PCB-degrading potential of some Gram-positive strains as well (genera Rhodococcus, Janibacter, Bacillus, Paenibacillus and Microbacterium) . Aerobic catabolic pathway for PCB degradation seems to be very similar for most of the bacteria and comprises four steps catalysed by the enzymes, biphenyl dioxygenase (BphA), dihydrodiol dehydrogenase (BphB), 2, 3-dihydroxybihenyl dioxygenase (DHBD) (BphC) and hydrolase (BphD).

Successful removal of pesticides by the addition of bacteria had been reported earlier for many compounds, including atrazine. Recent findings concerning pesticide degrading bacteria include the chlorpyrifos degrading bacterium Providencia stuartii isolated from agricultural soil and isolates Bacillus, Staphylococcus and Stenotrophomonas from cultivated and uncultivated soil able to degrade dichlorodiphenyltrichloroethane (DDT). Researches on bacterial strains that are able to degrade azo dyes under aerobic and anaerobic conditions have been extensively reported . Based on the available literature, it can be concluded that the microbial decolourization of azo dyes is more effective under anaerobic conditions. On the

other hand, these conditions lead to aromatic amine formation, and these are mutagenic and toxic to humans requiring a subsequent oxidative (aerobic) stage for their degradation. In this context, the combined anaerobic/aerobic biological treatments of textile dye effluents using microbial consortia are common in the literature. For exemple, Chaube et al. have used the mix consortia of bacteria consisting of Proteus sp., Pseudomonas sp. and Enterococcus sp. in biodegradation and decolorisation of dye. However, several researchers have identified single bacterial strains that have very high efficacy for removal of azo dyes, it is the case of Shewanella decolorations. In contrast to mixed cultures, the use of a pure culture has several advantages. These include predictable performance and detailed knowledge on the degradation pathways with improved assurance that catabolism of the dyes will lead to nontoxic end products under a given set of environmental conditions. Another advantage is that the bacterial strains and their activity can be monitored using culture-based or molecular methods to quantify population densities of the bacteria over time. Knowledge
of the popu- lation density can be extrapolated to quantitative analysis of the kinetics of azo dye decolor- ation and mineralization.

Heavy metals cannot be destroyed biologically (no"degradation", change in the nuclear structure of the element, occurs) but are only transformed from one oxidation state or organic complex to another. Besides, bacteria are also efficient in heavy metals bioremediation. Microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms, such as adsorption, uptake, methylation, oxidation and reduction. Reduction of metals can occur through dissimilatory metal reduction [40], where bacteria utilize metals as terminal electron acceptors for anaerobic respiration. In addition, bacteria may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance. For example, reduction of Cr(VI) to Cr(III) under aerobic or anaerobic conditions , reduction of Se(VI) to elemental Se, reduction of U(VI) to U(IV) and reduction of Hg(II) to Hg(0) . Microbial methylation plays an important role in heavy metals bioremediation, because methylated compounds are frequently volatile. For example, Mercury, Hg(II) can be biomethylated by a number of different bacterial species Alcaligenes faecalis, Bacillus pumilus, Bacillus sp., P. aeruginosa and Brevibacterium iodinium to gaseous methyl mercury . In addition to redox conversions and methylation reactions, acidophilic iron bacteria like Acidithiobacillus ferrooxidans and sulfur oxidizing bacteria are able to leach high concentrations of As, Cd, Cu, Co and Zn from contaminated
soils. On the other hand metals can be precipitated as insoluble sulfides indirectly by the metabolic activity of

sulphate reducing bacteria. Sulphate reducing bacteria are anaerobic heterotrophs utilizing a range of organic substrates with SO 2- as the terminal electron acceptor. Heavy metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure. This method of uptake is independent of the biological metabolic cycle and is known as biosorption or passive uptake. The heavy metal can also pass into the cell across the cell membrane through the cell metabolic cycle. This mode of metal uptake is referred as active uptake. Pseudomonas strain, characterized as part of a project to develop a biosorbent for removal of toxic radionuclides from nuclear waste streams, was a potent accumulator of uranium (VI) and thorium (IV).

Most works on pollutants bioremediation uses pure microbial cultures. However, the use of mixed microbial cultures is undoubtedly advantageous. Some of the best examples of enrich- ment cultures comprising several specific consortia involve the bioremediation. In the case of heavy metals removal, Adarsh et al. have used an environmental bacterial consortium to remove Cd, Cr, Cu, Ni and Pb from a synthetic wastewater effluent. For Cr(VI) removal we reported that the survival and stability of bacteria are better when they are present as a mixed culture, especially, in highly contaminated areas and in the presence of more than one type of metal . Indeed, the indigenous bacteria enriched from chromium contaminated biotopes, were able to remove Cr(VI) successfully in multi-contaminated heavy metal solution. A microbial consortium consisting of three bacterial Pseudomonas species originally obtained from dye contaminated sites was capable of decolorizing textile effluent and dye faster than the individual bacteria under static conditions.

**Sanitary Analysis of Waters**

Monitoring and detection of indicator and disease-causing mi- croorganisms are a major part of sanitary microbiology. Bacteria from the intestinal tract generally do not survive in the aquatic environment, are under physiological stress, and gradually lose their ability to form colonies on differential and selective media. Their die-out rate depends on the water temperature, the effects of sunlight, the populations of other bacteria present, and the chemical composition of the water. Procedures have been developed to attempt to “resuscitate” these stressed coliforms before they are identified using selective and differential media.

A wide range of viral, bacterial, and protozoan diseases result from the contamination of water with human fecal wastes. Although many of these pathogens can be detected directly, environmental microbiologists have generally used indicator organisms as an index of possible water contamination by human pathogens. Researchers are still searching for the "ideal" indicator organism to use in sanitary microbiology. The following are among the suggested criteria for such an indicator:

1. The indicator bacterium should be suitable for the analysis of all types of water: tap, river, ground, impounded, recreational, estuary, sea, and waste.
2. The indicator bacterium should be present whenever enteric pathogens are present.
3. The indicator bacterium should survive longer than the hardiest enteric pathogen.
4. The indicator bacterium should not reproduce in the contaminated water and produce an inflated value.
6. The assay procedure for the indicator should have great specificity; in other words, other bacteria should not give positive results. In addition, the procedure should have high sensitivity and detect low levels of the indicator. The testing method should be easy to perform.

7. The indicator should be harmless to humans.

8. The level of the indicator bacterium in contaminated water should have some direct relationship to the degree of fecal pollution.

Coliforms, including Escherichia coli, are members of the family Enterobacteriaceae. These bacteria make up approximately 10% of the intestinal microorganisms of humans and other animals and have found widespread use as indi- cator organisms. They lose viability in fresh water at slower rates than most of the major intestinal bacterial pathogens. When such "foreign" enteric indicator bacteria are not detectable in a specific volume (100 ml) of water, the water is considered potable [Latin potabilis, fit to drink], or suitable for human consumption. The coliform group includes E. coli, Enterobacter aerogenes, and Klebsiella pneumoniae. Coliforms are defined as facultatively anaerobic, gram-negative, nonsporing, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. The original test for coliforms that was used to meet this definition involved the presumptive, confirmed, and completed tests. The presumptive step is carried out by means of tubes inoculated with three different sample volumes to give an estimate of the most probable number

(MPN) of coliforms in the water. The complete process, including the confirmed and com- pleted tests, requires at least 4 days of incubations and transfers. Unfortunately the coliforms include a wide range of bacteria whose primary source may not be the intestinal tract. To deal with this difficulty, tests have been developed that allow waters to be tested for the presence of fecal coliforms. These are coliforms de- rived from the intestine of warm-blooded animals, which can grow at the more restrictive temperature of 44.5°C.

To test for coliforms and fecal coliforms, and more effectively recover stressed coliforms, a variety of simpler and more specific tests have been developed. These include the membrane filtration technique, the presence-absence (P-A) test for coliforms and the related Colilert defined substrate test for detecting both coliforms and E. coli.

The membrane filtration technique, has be- come a common and often preferred method of evaluating the microbiological characteristics of water. The water sample is passed through a membrane filter. The filter with its trapped bacteria is transferred to the surface of a solid medium or to an absorptive pad containing the desired liquid medium. Use of the proper medium allows the rapid detection of total coliforms, fecal coliforms, or fecal streptococci by the presence of their characteristic colonies. Samples can be placed on a less selective resuscitation medium, or incubated at a less stressful temperature, prior to growth under the final set of selective condi- tions. An example of a resuscitation step is the use of a 2 hour in- cubation on a pad soaked with lauryl sulfate broth, as is carried out in the LES Endo procedure. A resuscitation step often is needed with chlorinated samples, where the microorganisms are espe- cially stressed. Membrane filters have been widely used with water that does not contain high levels of background organisms, sediment, or heavy metals.

More simplified tests for detecting coliforms and fecal col- iforms are now available. The presence-absence test (P-A test) can be used for coliforms. This is a modification of the MPN pro- cedure, in which a larger water sample (100 ml) is incubated in a single culture bottle with a triple-strength broth containing lactose broth, lauryl tryptose broth, and bromcresol purple indicator. The P-A test is based on the assumption that no coliforms should be present in 100 ml of drinking water. A positive test results in the production of acid (a yellow color) and constitutes a positive presumptive test requiring confirmation.

To test for both coliforms and E. coli, the related Colilert defined substrate test can be used. A water sample of 100 ml is added to a specialized medium containing o-nitrophenyl-þ-D- galactopyranoside (ONPG) and 4-methylumbelliferyl-þ-D-glucuronide (MUG) as the only nutrients. If coliforms are present, the medium will turn yellow within 24 hours at 35°C due to the hydrolysis of ONPG, which releases o-nitrophenol. To check for E. coli, the medium is observed under long-wavelength UV light for fluorescence. When E. coli is present, the MUG is modified to yield a fluorescent product. If the test is negative for the presence of coliforms, the water is considered acceptable for human consumption. The main change from pre- vious standards is the requirement to have waters free of coliforms and fecal coliforms. If coliforms are present, fecal col- iforms or E. coli must be tested for.

Molecular techniques are now used routinely to detect coliforms in waters and other environments, including foods. 16 S rRNA gene-targeted primers for coliforms have been developed. Using these primers, it is possible to detect one colony- forming unit (CFU) of E. coli per 100 ml of water, if an eight-hour enrichment step precedes the use of the PCR amplification. This allows the differentiation of nonpathogenic and enterotoxigenic strains, including the shiga-toxin producing E. coli O157:H7.

In the United States a set of general guidelines for microbio- logical quality of drinking waters has been developed, including standards for coliforms, viruses, and Giardia. If un- filtered surface waters are being used, one coliform test must be run each day when the waters have higher turbidities. Other indicator microorganisms include fecal enterococci. The fecal enterococci are increasingly being used as an indicator of fecal contamination in brackish and marine water. In salt water these bacteria die back at a slower rate than the fecal coliforms, providing a more reliable indicator of possible recent pollution.

**Degradation by genetically engineered microorganisms**

Bioaugmentation and biostimulation are methods that can be applied to accelerate the recovery of polluted sites. In the late 1970s and early 1980s, bacterial genes encoding catabolic enzymes for recalcitrant compounds started to be cloned and characterized. Soon, many microbiologists and molecular biologists realized the potential of genetic engi- neering for addressing biodegradation. A genetically engineered microorganism (GEM) or modified microorganism (GMM) is a microorganism whose genetic material has been altered using genetic engineering techniques inspired by natural genetic exchange betweenmicroorganisms. These techniques are generally known as recombinant DNA technology. Genetically engineered microorganisms (GEMs) have shown potential for bioremediation of soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants. As soon as the prospect of releasing genetically modified microorganisms for bioremediation became a reality, much of the research effort in the field was aimed at biosafety and risk assessment.

There are at least four principal approaches to GEM development for bioremediation application. These include: 1) Modification of enzyme specificity and affinity; 2) Pathway construction and regulation; 3) Bioprocess development, monitoring and control; 4) Bioaffinity bioreporter sensor applications for chemical sensing, toxicity reduction and end point analysis.

**Genetically engineered microorganisms**

Molecular biology offers the tools to optimize the biodegradative capacities of microorgan- isms, accelerate the evolution of "new" activities, and construct totally "new" pathways through the assemblage of catabolic segments from different microbes. Genes responsible for degradation of environmental pollutants, for example, toluene, chloro- benzene acids, and other halogenated pesticides and toxic wastes have been identified. For every compound, one separate plasmid is required. It is not like that one plasmid can degrade all the toxic compounds of different groups. The plasmids are grouped into four categories: 1) OCT plasmid which degrades, octane, hexane and decane; 2) XYL plasmid which degrades xylene and toluenes, 3) CAM plasmid that decompose camphor and 4) NAH plasmid which degrades naphthalene.

The potential for creating, through genetic manipulation, microbial strains able to degrade a variety of different types of hydrocarbons has been demonstrated by Friello et al. They successfully produced a multiplasmid-containing Pseudomonas strain capable of oxidizing aliphatic, aromatic, terpenic and polyaromatic hydrocarbons.

Pseudomonas putida that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombinating parts of CAM and OCT developed by conjugation could degrade camphor, octane, salicylate, and naphthalene and could grew rapidly on crude oil because it was capable of metabolizing hydrocarbons more efficiently than any other single plasmid. This product of genetic engineering was called as superbug (oil eating bug). The plasmids of P. putida degrading various chemical compounds are TOL (for toluene and xylene), RA500 (for 3, 5-xylene) pAC 25 (for 3-cne chlorobenxoate), pKF439 (for salicylate toluene). Plasmid WWO of P. putida is one member of a set of plasmids now termed as TOL plasmid. It was the first living being to be the subject of an intellectual property case. At that point, it seemed that molecular techniques, either through plasmid breeding or sheer genetic engineering, could rapidly produce microbes with higher catalytic abilities, able to basically degrade any environmental pollutant. Reports on the degradation of environmental pollutants by genetically engineered microorganisms are focused on genetically engineered bacteria using different genetic engineering technologies: Pathway modification, modification of substrate specificity by Comamonas testosteroni VP44. The application of genetic engineering for heavy metals removal has aroused great interest. For example, Alcaligenes eutrophus AE104 (pEBZ141) was used for chromium removal from industrial wastewater and the recombinant photosynthetic bacterium, Rhodopseudomonas palustris, was constructed to simultaneously express mercury transport system and metallothionein for Hg2+ removal from heavy metal wastewater.

For polychlorinated biphenyls degradation, chromosomally located PCB catabolic genes of R. eutropha A5, Achromobacter sp. LBS1C1, and A. denitrificans JB1 were transferred into a heavy metal resistant strain R. eutropha CH34 through natural conjugation. Genetic engineering of endophytic and rhizospheric bacteria for use in plant associated degradation of toxic compounds in soil is considered one of the most promising new technol- ogies for remediation of contaminated environmental sites. To select a suitable strain for gene recombination and inoculation into the rhizosphere, there are three criteria that has been recommended: first, the strain should be stable after cloning and the target gene should have a high expression, second, the strain should be tolerant or insensitive to the contaminant; and third, some strains can survive only in several specific plant rhizosphere. Many bacteria in the rhizosphere show only limited ability in degrading organic pollutants. With the development of molecular biology, the genetically engineered rhizobacteria with the contam- inant-degrading gene are constructed to conduct the rhizoremediation [58]. Examples about the molecular mechanisms involved in the degradation of some pollutants such as trichloro- ethylene (TCE) and PCBs has been studied.For heavy metals, Sriprang et al. introduced Arabidopsis thaliana gene for phytochelatin synthase (PCS; PCSAt) into Mesorhizobium huakuii subsp. rengei strain B3 and then established the symbiosis between M. huakuii subsp. rengei strain B3 and Astragalus sinicus. The gene was expressed to produce phytochelatins and accumulate Cd2+, under the control of bacteroid- specific promoter, the nifH gene. Finally, the use of GEM strains as an inoculum during seeding would preclude the problems associated with competition between strains in a mixed culture. However, there is considerable controversy surrounding the release of such genetically engineered microorganisms into the environment, and field testing of these organisms must therefore be delayed until the issues of safety and the potential for ecological damage are resolved.

**ENVIRONMENTAL MICROBIOLOGY - 18MBU403**

**Possible Questions – Unit V**

1. Write a note on pesticides.
2. Give an account of on MPN test.
3. Sketch a note on GMO and their impact.
4. Describe in detail about membrane filter technique
5. Discuss in detail about heavy metal chelation
6. Write short notes on treatment and safety of drinking water.
7. Give an account on biosurfactants
8. Comment on the principles and biodegradation of common pesticides.
9. Write a note on the principles and biodegradation of hydrocarbons, oil spills.

10.Explain about microbes in bioremediation.

| | Unit V | | | | | |
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| **Sno** | **Question** | **Option 1** | **Option 2** | **Option 3** | **Option 4** | **Answer** |
| 1 | Most of the indicator oranisms for detection of disease occurrence level in drinking water belongs to which of the following microroganisms group | Actinobacteria | Bacilli | Coliform | Firmicutes | Coliform |
| 2 | Which among the following microbes is not a prime concern for deterioration of water quality in drinking water | Legionella | Shigella | Vibrio parahaemolyticus | Vibrio vulnificus | Shigella |
| 3 | Which of the following test is used as preusmptive test for enumeration of coliform in water samples? | Most probable number | Heterocoliformcount | Aerobic colony count | colony forming unit | Most probable number |
| 4 | Which water is otherwise known as portable water? | Raw | Irrigation | Drinking | Surface | Drinking |
| 5 | The environment which has been modified by human activities is called | Natural | Urban | Anthropogenic | Modern | Anthropogenic |
| 6 | The top layer of the lake is called as________ | Thermocline | Epilimnion | Thermonion | Hypolimnion | Epilimnion |
| 7 | The presence of high coliform counts in water indicates___________ | Contamination by human wastes | Phosphorous Contamination | Decreased biological oxygen demand | Hydrocarbon Contamination | Contamination by human wastes |
| 8 | Oxidation ponds are shallow ponds, generally designed at the depth of | 2 to 40 feet | 4 to 6 feet | 1 to 3 feet | 5 to 8 feet | 2 to 40 feet |

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| 9 | Water testing relies on the detection of certain indicator organisms known as | acid-fast bacteria | bacteroids | coliforms | dinoflagellates | coliforms |
| 10 | What would be a typical number for the number of Escherichi coli cells allowed in drinking water samples? | 1000 per 100 mL | 10 per mL | 0 per 100 mL | 10000 per mL | 0 per 100 mL |
| 11 | For most bacteria, the optimum pH for growth lies between | 6.5-7.5 | 3.5-4.5 | 4.5-5.5 | 5.5-6.5 | 6.5-7.5 |
| 12 | Generation time of Escherichia coli is | 20 minutes | 20 hours | 20 days | 200 hours | 20 minutes |
| 13 | An organism that expends energy to grow in a habitat with a low water activity in order to maintain internal solute concentrations to retain water is | osmotolerant | acidophile | aerotolerant anaerobe | alkalophile | osmotolerant |
| 14 | Bacteria and fungi multiply best | below 16°C | between 16-38°C | below 0°C | above 38°C | between 16-38°C |
| 15 | Organisms, using organic compounds as electron donors are called | lithotrophs | phototrophs | chemotrophs | organotrophs | organotrophs |
| 16 | Which of the following organisms has more tolerance for acidic pH (lower pH)? | Yeast and moulds | Bacteria | E. coli | virus | Yeast and moulds |
| 17 | The term facultative anaerobe refers to an organism that | doesn't use oxygen but tolerates it | is killed by oxygen | uses oxygen when present or grows without oxygen when absent | requires less oxygen than is present in air | uses oxygen when present or grows without oxygen when absent |
| 18 | The microorganisms that grow best in a low-oxygen environment is called an | aerobe | anaerobe | facultative | microaerophile | microaerophile |

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| 19 | Bacteria of genus Nitrosomonas use ___________ as their electron source. | ammonia | H2S | succinate | light | ammonia |
| 20 | In the human disease cholera, what is it that actually ends up killing the victim? | Faulty carrier proteins | Dehydration and loss of nutrients | oo little water in the food stream | the toxin produced by the bacterium | Dehydration and loss of nutrients |
| 21 | Which of the following is generally not referred to the sewerage system? | Sanitary sewers | Storm sewers | Combined sewers | Solid sewers | Solid sewers |
| 22 | For rapid decomposition by microbes, the substrate should have a C/N ratio of | 10 to 20 | 20-30 | 30-40 | 60-80 | 30-40 |
| 23 | Most of the indicator oranisms for detection of disease occurrence level in drinking water belongs to which of the following microroganisms group | Actinobacteria | Bacilli | Coliform | Firmicutes | Coliform |
| 24 | Which among the following microbes is not a prime concern for deterioration of water quality in drinking water | Legionella | Shigella | Vibrio parahaemolyticus | Vibrio vulnificus | Shigella |
| 25 | Which of the following test is used as preusmptive test for enumeration of coliform in water samples? | Most probable number | Heterocoliformcount | Aerobic colony count | colony forming unit | Most probable number |
| 26 | Which water is otherwise known as portable water? | Raw | Irrigation | Drinking | Surface | Drinking |
| 27 | The environment which has been modified by human activities is called | Natural | Urban | Anthropogenic | Modern | Anthropogenic |

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| 28 | _______ are mixed cultures of naturally occuring beneficial microbes used to degrade contaminants, increase quality of soil etc | Enhanced microorganisms | Efficient microorganisms | Effective microorganisms | Good microorganisms | Effective microorganisms | |
| 29 | ________ is a process of bioremediation used to improve quality of underground water | Bioaccumulation | Biostimulation | Bioventing | Biodegradation | Biostimulation and bioleaching | |
| 30 | _________ bioremediation involves treating the contaminated material at the site | *Ex situ* | *In situ* | *In vivo* | *In vitro* | *In situ* | |
| 31 | _____________ is a method of composting by piling biodegradable waste in long rows | Windrow method | Window method | Landfilling | In-vessel composting | Windrow method | |
| 32 | Addition of archaea or bacterial cultures to speed up the rate of degradation of a contaminant | Bioaugumentation | Bioriching | Biobleaching | Biomediation | Bioaugumentation | |
| 33 | An engineered device or system that supports a biologically active environment | Incubator | Shake flask | Biofilter | Bioreactor | Bioreactor | |
| 34 | Bacteria used in bioleaching | *Vibrio cholerae* | *Salmonella typhi* | *Acidithiobacillus ferrooxidans* | *Lactobacillus caseii* | *Acidithiobacillus ferrooxidans* | |

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| 35 | Biostimulation is the process of | Organic farming | Enhancing growth conditions of microbes | Metal ore leaching | Isolation & Screening | Enhancing growth conditions of microbes | |
| 36 | Common metal contaminants of sea | Magnesium | Aluminium | Copper | Zinc | copper | |
| 37 | Fungi that have the ability to degrade an extremely diverse range of toxic environmental pollutants | *Aspergillus niger* | *Mucor* | *Fusarium* | *Phanaerochaete chrysosporium* | *Phanaerochaete chrysosporium* | |
| 38 | Intracellular accumulation of environmental pollutants such as heavy metals by living organisms is | Bioriching | Biobleaching | Biomediation | Bioaccumulation | Bioaccumulation | |
| 39 | Oldest form of waste treatment/disposal | Burning | Shredding | Vermicomposting | Landfill | Landfill | |
| 40 | Process of supplying oxygen in situ to microbes in contaminated soil at low flow rates | Bioenhancing | Bioventing | Biomediation | Bioriching | Bioventing | |
| 41 | Technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site | Bioremediation | Disposal | Sewage treatment | Waste removal | Bioremediation | |
| 42 | The use of living microorganism to degrade environment pollutants is called___________ | microremediation | nanoremediation | bioremediation | all of these | bioremediation | |

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| 43 | The process of converting environmental polltants into harmless products by naturally occuring microbes is called __________ | bioextraction | microbial extraction | biofiltration | bioleaching | bioleaching | |
| 44 | Ex situ bioremediation involves the | Degradation of pollutants by microbes directly | Removal of pollutants and collection at a place to faxilitate microbial degradation | Degradation of pollutants by genetically | bioremediation | Removal of pollutants and collection at a place to facilitate microbial degradation | |
| 45 | Which of the following is not an application of bioremediation | Treating oil spills by oil-eating microbes | Sewage treatment by bacteria | Burning of petroleum products floatng on the surface of the sea | Removal of grease by bacteris that digest FOG | Burning of petroleum products floatng on the surface of the sea | |

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| 46 | Which of the following is not true with respect to the advantages of bioremediation | Inexpensive | Cost effective | Contaminants are not completely destroyed | Environment friendly and natural process | Contaminants are not completely destroyed |
| 47 | Bioremediation is the process of removing contaminants or pollutants from the environment to return it to its original state through the use of _________ mechanisms. | Chemical | Abiotic | biological | Homeostatic | Biological |
| 48 | The addition of known active microbes to soil or water with the purpose of accelerating microbial processes is called ___________ | Biodegradation | Bioremediation | Bioaccentuation | Bioaugmentation | Biodegradation |
| 49 | Which microorganisms are most commonly used bacteria for bioremediation? | Bacillus | Klebsiella | Staphylococcus | Streptococcus | Bacillus |
| 50 | Advanced treatment is generally used to treat waste water to | remove coarse solids | remove settleable solids | reduce BOD | remove additional objectionable substances | remove additional objectionable substances |
| 51 | The concept of putting microbes to help clean up the environment is called | pasteurization | bioremediation | fermentation | biolistics | bioremediation |

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| 52 | Treatment of municipal water supplies is based upon | coagulation, filtration, chlorination | chlorination, filtration, coagulation | filtration, coagulation, chlorination | coagulation, chlorination, filtration | coagulation, filtration, chlorination |
| 53 | which of the following is generally not referred to the sewerage system? | Sanitary sewers | Storm sewers | Combined sewers | Solid sewers | Solid sewers |
| 54 | Schmutzdecke is a hypogeal biological layer formed on surface of slow sand filter by | Fungi | Bacteria | Protozoa | Algae | Bacteria |
| 55 | Sludge conditioning is accomplished by which of the following | Thickening | Elutriation | Chemical conditioning | Diluting with water | Thickening |
| 56 | A dense bacterial population caught in a tangled web of fibers sticking to a surface describes | biodisc | coagulation | the membrane filter technique | a biofilm | a biofilm |
| 57 | The filtering medium of the tank becomes coated with a microbial flora, the ___________ film | Biofilm | Zooglocal film | Neustonic | Algal bloom | Zooglocal film |
| 58 | The most commonly efficient substrate used as a carbon source indenitrification during sewage treatement is | Methanol | Oxygen | Glucose | Sucrose | Methanol |
| 59 | The optimum rate of relative humidity for the survival of the most microorganisms is | 40-80% | 60-80% | 50-80% | 30-80% | 40-80% |
| 60 | Which of the following is not an aerobic process? | Activated sludge process | Sludge digestion | Trickling filter | Oxidation pond | Trickling filter |