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Instruction Hours / week: L: 5 T: 0 P: 0

Marks: Internal: 40 External: 60 Total: 100

End Semester Exam: 3 Hours

**SCOPE**

Microbes and environment, classification occurrence, distribution, diversity and ecological importance, characteristics of protists, prokaryotes and viruses. Photoautotrophs, chemolithotrophs, organotrophs, parasites and symbionts.

**OBJECTIVES**

This course provides a comprehensive overview of biogeochemical processes relevant to environmental scientists and engineers mediated by microorganisms. Water born pathogens and water borne disease microbial standards of water quality, biogenic pollution, microbial toxins, air borne microbes, microbial dispersal through air and water estimation of microbial biomass.

**UNIT - I**

Aero biology : Droplet nuclei, aerosol, assessment of air quality-solid, liquid impingement methods. Air borne diseases – causative agent – viruses, bacteria and fungi and preventive measures.

**UNIT - II**

Aquatic microbiology: Water ecosystems - types of fresh water (ponds, lake, streams) marine habitats (estuaries, mangroves, deep sea, hydrothermal vents, salt pans, coral reefs). - Food chain. Potability of water- microbial assessment of water quality- water purification - Major water borne diseases and their control measures.

**UNIT - III**

Waste treatment: Types of wastes - solid and liquid wastes characterization; treatments- physical, chemical, biological; solid waste treatment – saccharification, gasification, composting. Liquid waste treatment- trickling- activated sludge - oxidation pond- oxidation ditch.

**UNIT - IV**

Bioremediation: Utilization for solid wastes - (SCP, mushroom, yeast): fuel (ethanol, methane) fertilizer (composting). Subterranean microbes and bioremediation. Waste disposal-solid, liquid, sewage.

**UNIT - V**

Positive and negative roles of microbes in environment: biodegradation of recalcitrant compounds - lignin - pesticides; bioaccumulation of metals and detoxification –biopesticides, biodeterioration of paper, leather, wood textiles, metal corrosion. Mode of deterioration- organisms involved -its disadvantages- mode of prevention. GMO and their impact.

**TEXT BOOKS**

1. Joseph C. Deniel, 1996, Environmental aspects of microbiology, British Sun Publication, Chennai.
2. Abbasi, S.A. 1998. Environmental pollution and its control. Cogent International publishers, Pondicherry.
3. Keya Sen and Nicholas J. Ashbolt 2010. Environmental Microbiology: Current Technology and Water Applications.

## REFERENCE

1. Atlas, R.M. and Bartha, R. 1993. Microbial ecology, fundamentals and applications, 3<sup>rd</sup> Edition. The Benjamin/Cummings Publishing Co., New Delhi.
2. Mitchell, R. and Gu J.D. 2010. Environmental Microbiology. 2<sup>nd</sup> Edition, Wiley-Blackwell. John Wiley and Sons Publications, Canada.
3. Raina, M., Maier, Ian L. Pepper and Charles P. Gerba, 2008. Environmental Microbiology. 2<sup>nd</sup> Edition. Academic Press. New York.
4. Ralph Mitchell and Ji-Dong Gu, 2010. Environmental Microbiology. 2<sup>nd</sup> Edition. Wiley-Blackwell, A John Wiley and Sons, Inc., Publications.

**III B. Sc Microbiology –Environmental Microbiology  
Unit I**

**LECTURE PLAN**

LECTURE PLAN - UNIT -1			
<b>S. no</b>	<b>Lecture duration(Hr)</b>	<b>Topics covered</b>	<b>Supporting materials</b>
1	1	Introduction to Environmental Microbiology	T1 1-4
2	1	Structure and function of Ecosystems	R1 18-37
3	1	Terrestrial environment soil profile and soil micro flora	R2 647-650
4	1	Aquatic environment- Micro flora of fresh water and Marine Habits	R2 644-650
5	1	Aeromicroflora and dispersal of microbes	T1 343-350
6	1	Microbes in human and animal body	R2 583-585, R2 699-704
7	1	Extremophiles	R2 624
8	1	Microbial succession in decomposition of plant organic matter	T1 188-189
<b>Textbooks :</b>		T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4 <sup>th</sup> Edition.	
<b>Reference books:</b>		R1-Fudamentals of ecology– Odum Ep & Barret GW. 5 <sup>th</sup> Edition R2- Microbiology. Prescott, Harley & Klein.6 <sup>th</sup> Edition	
<b>Website:</b>		-	
<b>Journals:</b>		-	

## Unit I

### Question

### Option 1

### Option 2

Microbes in air can be enumerated by _____	Settle plate method	Pour plate method
_____ is a source of contamination of air borne diseases	Sneezing	Coughing
Effective air sanitizing is done by _____	Gamma radiation	UV radiation
The principle involved in the air sampler Hesses tube is _____	Centrifugal action	Settling under gravi
The principle involved in Litton air sampler is _____	Centrifugal action	Electrostatic precipi
Who first showed that microorganisms could occur as airborne _____	Koch	Spallanzani
Droplet nuclei are significant in the transmission of diseases of _____	Digestive system	Nervous system
Air borne infections are transmitted mainly by _____	Aerobes from person	Inhaling spores or h
The atmospheric layer nearest to the earth is _____	Troposphere	Stratosphere
Air mainly acts a _____ medium for microorganisms	transport or dispersal	Growth medium
Usually _____ are present at very high altitudes.	Spores	Vegetative cells
The air found inside the building is referred to as _____	Indoor air	out door air
The common problem associated with Nosocomial pathogens is _____	Antibiotic resistance	Dysentery
The filtering material used in granular filter is _____	Activated charcoal	Glass wool
Of the different atmospheric layers _____ is characterized	Troposphere	Stratosphere
The type of isolation in which susceptible persons are separated from _____	Protective isolation	cubic isolation
_____ may act as more potential agents of infectious dis	Droplet nuclei	Dust particles
_____ are relatively more abundant than the veg	Spores	Infectious dust
_____ is an occupational disease	Brucellosis	Pulmonary disease
_____ removes almost all microorganisms from atmospl	Rainfall	pH
Spores of _____ travel over a thousand kilomet	Clostridium perfringe	Puccinia graminis
Farmer's lung caused by exposure to spores of thermophilic ____	Fungi	Bacteria
HEPA filters are typically rated as _____ effective in remov	90	99.92%
In Lemon sampler air is drawn at the rate of _____ per	20-25litre	25-30litre
Laminar airflow developed by _____.	Whittaker	Whitfield
Lilton large volume air sampler is an example for _____	Sieve sampler	Impingement
Of the different atmospheric layers _____ is characterized b	Troposphere	Stratosphere
Slit sampler can collect upto _____% of the water droplet part	85%	100%
The amount of carbondioxide present in the atmosphere is near	0.02%	0.03%
The dominant genera of common saprophytic fungi in indoor ai	Aspergillus	Fusarium
Viruses survive in the atmosphere at low temperature from _____	8 to 32°C	7 to 24°C
_____ is a seasonal disease that can be deadly when becom	Tuberculosis	Leprosy
_____ air sampler is used in electrostatic precipitation of r	Impinger	Orange
_____ is a severe respiratory disease caused by bacteria	Aspergillosis	Scarlet fever
_____ is an opportunistic fungal disease of human cause	Sporidiasis	Penicilliosis
_____ is transmitted to humans by inhalation of faec	Candidiasis	Cryptococcosis

According to ambient air quality standards in India, amount of _____	200 $\mu\text{gm}^{-3}$	400 $\mu\text{gm}^{-3}$
Air doesn't have _____ flora	Indigenous	Endogenous
Aspergillosis	Human	Soil
Cryptococcosis is caused by	<i>C.neoformans</i>	<i>C.licheniformis</i>
Efficiency of HEPA filter	75%	90%
HEPA is an	Water filter	Soil filter
HEPA stands for	High Efficiency Particulate	High Energy Particulate
Optimum rate of relative humidity for the survival of most microorganisms	10-20%	30-60%
Percentage of oxygen in atmospheric air	40.9	60.9
Residue of solid material left after drying up of a droplet is known as	Mucus	Karyon
Size of a droplet nuclei	0.1-0.4 $\mu\text{m}$	1-4 $\mu\text{m}$
Ozone is formed in the upper atmosphere by a photochemical reaction of	Ultraviolet solar radiation	Infra red radiation
Identify the term that describes an environment completely free of microorganisms	Antibiotic	Asepsis
The commonest genera of fungi in indoor air are	<i>Saccharomyces</i>	<i>Penicillium</i>
The dominant microflora of outside air are	Fungi	Bacteria
Transmission of _____ is mainly by inhaling the dust containing	Cholera	Anthrax
_____ is a syndrome caused by inhalation of large concentrations of	Bronchitis	Cold
Mycobacterium tuberculosis was first discovered by	Robert Koch	Edward Jenner
Streptococcus pneumoniae was isolated by	Robert Koch	Edward Jenner
Acid fast bacteria?	cell walls with mycolic acid	exotoxin production
MMR protects against what three diseases?	measles, malaria, and monkey pox, mumps	
Diphtheria is caused by	Corynebacterium	Staphylococcus
An example for common air borne epidemic disease	Influenza	Typhoid
Diphtheria bacillus is otherwise known as	Fried-Land's bacillus	Kleb's hofmann's bacillus















**Y (KARPAGAM ACADEMY OF HIGHER EDUCATION)**

**DEPARTMENT OF MICROBIOLOGY**

**MENTAL MICROBIOLOGY 15MBU504**

**III BSc Microbiology**

<b>Option 3</b>	<b>Option 4</b>	<b>Answer</b>
Spread plate method	All above	Settle plate method
Talking	All above	All above
Beta radiation	Nano radiation	UV radiation
Filtration	impingement	Settling under gravity
Filtration	impingement	Centrifugal action
Schwann	Pasteur	Pasteur
Reproductive system	Respiratory system	Respiratory system
Drinking contamination	Objects such as handkerchiefs that are common	Aerobes from person
Lithosphere	None of the above	Troposphere
sterilised medium	none of the above	transport or dispersal
Mycelium	viruses	Mycelium
sterilised air	contaminated air	Indoor air
Diarrhoea	Fever	Antibiotic resistance
ore	calcium carbonate	Activated charcoal
Lithosphere	None of the above	Troposphere
source isolation	none of the above	Protective isolation
Aerosols	none of the above	Droplet nuclei
Aerosols	Droplets	Spores
Pneumonitis	Meningitis	Brucellosis
Chemicals	Mutagenesis	Rainfall
Sarcina lutea	Micrococcus luteus	Clostridium perfringens
Actinomycetes	Viruses	Actinomycetes
99.97%	90.99%	99.97%
30-35litre	35-40litre	20-25litre
Tyndall	Koch	Whitfield
Electrostatic precipitation	Centrifugal action	Electrostatic precipitation
Lithosphere	Atmosphere	Troposphere
95%	75%	85%
0.04%	0.05%	0.04%
Penicillium	Mucor	Penicillium
6 to 18°C	2 to 6°C	6 to 18°C
Food poisoning	Influenza	Influenza
Sutton small volume	Litton large volume	Litton large volume
Tuberculosis	Tetanus	Tuberculosis
Aspergillosis	Candidiasis	Aspergillosis
Sporidiasis	Bacteraemia	Cryptococcosis

600 $\mu\text{gm}^{-3}$	800 $\mu\text{gm}^{-3}$
Exogenous	Subgenous
Industries	Vehicles
<i>C.pseudopodis</i>	<i>C.parvum</i>
95%	99.97%
Air filter	Smoke arrester
High Emission Particulate	High Efficient Polar Aerosol
40-80 %	80-100%
75.9	20.9
Oocyst	Droplet nuclei
4-10 $\mu\text{m}$	10-40 $\mu\text{m}$
Visible light	Gamma rays
Antisepsis	Probiotic
<i>Rhizopus</i>	<i>Mucor</i>
Algae	Virus
Typhoid	Dysentery
Hypersensitivity pneumonia	Pneumonitis
Louis Pasteur	None of these
Antony von Leewenhook	Louis Pasteur
skin infections that can lead to septicimia	
measles, mumps, rubella	none of the above
Streptococcus	None of these
Encephalitis	Malaria
Fuchs bacillus	Koch's bacillus

200 $\mu\text{gm}^{-3}$
Indigenous
Both I & II
<i>C.neoformans</i>
99.97%
Air filter
High Efficiency Particulate Air
40-80 %
20.9
Droplet nuclei
1-4 $\mu\text{m}$
Ultra violet solar radiation
Asepsis
<i>Penicillium</i>
Fungi
Anthrax
Hypersensitivity pneumonia
Robert Koch
Louis Pasteur
cell walls with mycolic
measles, mumps, rubella
Corynebacterium
Influenza
Klebsiella hofmanni bacillus















## UNIT - 1

### 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.

##### Diphtheria:

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

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*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 bronchopneumonia. *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.

### **Coccidioidomycosis:**

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:**

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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 Puccinia graminis travel over a thousand kilometers. However, the transmission of animal diseases is not usually important in outside 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 of airborne 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.

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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, 1-4 $\mu$ m, 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 saliva 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  $\mu$ m 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 and 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 from 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 consists 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, whether solid or liquid. This layer can be anywhere from 1  $\mu\text{m}$  to several meters thick depending upon weather conditions. Still conditions 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 particles 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 nearby 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) germinates and grows, when dispersed on a compatible surface, gaining the metabolic efficiency, it perishes on coming in contact with an incompatible surface.

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## **Bioaerosol:**

The bioaerosol are the atmospheric particles, mist or dust of  $\mu\text{m}$  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  $\mu\text{m}$  in diameter and are classified on the basis of their size. The smaller the particle  $<0.1 \mu\text{m}$  in diameter are considered to be in the nuclei mode, those ranging from 0.1-2  $\mu\text{m}$  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 mode 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 as 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 be 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 biosolid 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

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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.

### **(a) 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  $8 \times 10^3$  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.

## **(b) 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.

## **(c) 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 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 well 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 as 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 neutral 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:**

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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 longer 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 temperature 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 petri 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

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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 consists of an air-sealed drum that contains a clockwork rotating disc 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 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.



**III B. Sc Microbiology –Environmental Microbiology  
Unit II**

**LECTURE PLAN**

LECTURE PLAN - UNIT -2			
<b>S. no</b>	<b>Lecture duration(Hr)</b>	<b>Topics covered</b>	<b>Supporting materials</b>
1	1	Microbes Interactions	T1 70-101
2	1	Microbe plant interaction Symbiotic	T1 119-123
3	1	Microbe plant interaction-Non Symbiotic	T2 109-118
4	1	Microbes in Ruminants	R2 583-585
5	1	Nematophagus fungi	T1 167-169
6	1	Symbiotic luminescent bacteria	T2 170-171
<b>Textbooks :</b>		T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4 <sup>th</sup> Edition.	
<b>Reference books:</b>		R1-Fudamentals of ecology– Odum Ep & Barret GW. 5 <sup>th</sup> Edition R2- Microbiology. Prescott, Harley & Klein.6 <sup>th</sup> Edition	
<b>Website:</b>		-	
<b>Journals:</b>		-	

## Unit II

### Question

### Option 1

### Option 2

Average salinity of seawater	15 ppt	25 ppt
Bacteriological examination of water usually employs	Total count	Multiple tube method
Ecological region at the lowest level of a body of water such as	Limnetic zone	Littoral zone
In a lake the combined littoral and limnetic zone is known as	Profundal zone	Euphotic zone
Main photosynthetic body of the lake	Littoral zone	Limnetic zone
Microorganisms found attached to the rock surface are referred	Epiphyton	Episammon
Profundal zone is	Open surface of water	Sub-surface zone
Which of the following can be seen in marine environment?	Halophiles	Barophiles
Which of the following is not common in marine environment?	Luminous bacteria	Psychrophilic bacteria
Zone near shore area where sunlight penetrates the sediment and	Littoral zone	Limnetic zone
which of the following microorganism grows well at temperature	Halobacterium	Methanococcus jannaschii
Most of the indicator organisms for detection of disease occurrence	Actinobacteria	Bacilli
Which among the following microbes is not a prime concern for	Legionella	Shigella
Which of the following test is used as presumptive test for enteric	Most probable number	Heterocolidiform count
Which water is otherwise known as portable water?	Raw	Irrigation
The environment which has been modified by human activities is called	Natural	Urban
The top layer of the lake is called as _____	Thermocline	Epilimnion
The presence of high coliform counts in water indicates _____	Contamination by human	Phosphorous Contamination
Oxidation ponds are shallow ponds, generally designed at the depth of	2 to 40 feet	4 to 6 feet
Water testing relies on the detection of certain indicator organisms like	acid-fast bacteria	bacteroids
What would be a typical number for the number of Escherichia coli cells	1000 per 100 mL	10 per mL
Which is not true about Anabaena and Nostoc?	Filamentous	Nitrogen fixing
Cyanobacteria are _____	Photoheterotrophs	Chemotrophs
Which one of the following was Gram negative, chemolithotrophic and	Siderococcus	E.coli
An organism that is osmophilic and has specific requirements for sodium	Halophile	Basophile
Diagnosis of carrier of salmonella typhi may be shown by	Fecal culture	Bile culture
Alkaliphiles grow at pH value between	1 to 6	6 to 9
The micro-organisms grow at high salinity are	Osmophiles	Halophiles
Which of the following microorganism use H <sub>2</sub> S as the electron donor	Chromatium	Chlorobium
Which of the following can be seen in marine environment?	halophiles	barophiles
Lakes in which mixing of water is incomplete are referred to as	holomictic	hypomictic
The open area found above the light compensation level in a lake is called	littoral zone	limnetic zone
In a lake the combined littoral and limnetic zone is known as	profundal zone	euphotic zone
The best example for total coliforms is _____	E.coli	Salmonella
Bacteria, as a group, are responsible for	nitrogen oxidation	sulfur oxidation
What are Blue-Green bacteria called?	Acquaobacteria	Cyanobacteria
The Archaea include all of the following except	methanogens	halophiles
What is the primary source of food for marine life?	Phytoplankton	Zooplankton

Autotrophic bacteria are those which	make their own food	form a long chain gly
The amount of oxygen dissolved in hypolimnion water in the winter	greater	lesser
Copper is used in water treatment as a	Disinfectant	Indicator
Marine ecosystems cover approximately _____ of the Earth's st	7%	71%
The filtering medium of the tank becomes coated with a microb	Biofilm	Zoogloeal film
Zone near shore area where sunlight penetrates the sediment an	Littoral zone	Limnetic zone
Zoogloeal film formed in the trickling filter consists of	Bacteria	Algae
_____ is an underground layer of water-bearing perme	Bore well	Ground well
Study of water flow in aquifers and the characterization of aqu	Hydrogeology	Hydrology
_____ is the used for seed treatment of groundnut	<i>Azospirillum</i>	<i>Azotobacter</i>
Which of the following is generally not referred to the sewerage syst	Sanitary sewers	Storm sewers
For rapid decomposition by microbes, the substrate should have a C/	10 to 20	20-30
For most bacteria, the optimum pH for growth lies between	6.5-7.5	3.5-4.5
Generation time of <i>Escherichia coli</i> is	20 minutes	20 hours
An organism that expends energy to grow in a habitat with a low wa	osmotolerant	acidophile
Bacteria and fungi multiply best	below 16°C	between 16-38°C
Organisms, using organic compounds as electron donors are called	lithotrophs	phototrophs
Which of the following organisms has more tolerance for acidic pH (	Yeast and moulds	Bacteria
The term facultative anaerobe refers to an organism that	doesn't use oxygen but	is killed by oxygen
The microorganisms that grow best in a low-oxygen environment is (	aerobe	anaerobe
Bacteria of genus <i>Nitrosomonas</i> use _____ as their electron so	ammonia	H <sub>2</sub> S
In the human disease cholera, what is it that actually ends up killing	Faulty carrier proteins	Dehydration and loss













**Y (KARPAGAM ACADEMY OF HIGHER EDUCATION)**  
**DEPARTMENT OF MICROBIOLOGY**  
**MENTAL MICROBIOLOGY 15MBU503**  
 III BSc Microbiology

<b>Option 3</b>	<b>Option 4</b>	<b>Answer</b>
35 ppt	45 ppt	35 ppt
Membrane filters count	Plate count	Multiple tube method
Profoundal zone	Benthic zone	Benthic zone
Metalimnion	Epilimnion	Epilimnion
Profoundal zone	Pelagic zone	Limnetic zone
Epixylon	Epilithon	Epilimnion
Deepest zone	Side zone	Deepest zone
Psychrophiles	Hydrophiles	Halophiles
Thermophilic bacteria	Barophilic bacteria	Luminous bacteria
Pelagic zone	Profoundal zone	Littoral zone
Pyrococcus furiosus	Bacillus	Pyrococcus furiosus
Coliform	Firmicutes	Coliform
Vibrio parahaemolyticus	Vibrio vulnificus	Shigella
Aerobic colony count	colony forming unit	Most probable number
Drinking	Surface	Drinking
Anthropogenic	Modern	Anthropogenic
Thermionion	Hypolimnion	Epilimnion
Decreased biological oxygen demand	Hydrocarbon Contamination	Contamination by humans
1 to 3 feet	5 to 8 feet	2 to 40 feet
coliforms	dinoflagellates	coliforms
0 per 100 mL	10000 per mL	0 per 100 mL
Cyanobacteria	Symbiotic	Symbiotic
Prototrophs	Photoautotrophs	Photoautotrophs
Spirillum	Mycoplasmas	E.coli
Barophile	Xerophile	Halophile
Urine culture	All of these	All of these
1 to 11	7 to 12	7 to 12
Both a and b	None of these	Both a and b
Both a and b	Rhodospirillum rubrum	Both a and b
psychrophiles	all the above	all the above
meromictic	amictic	holomictic
profundal zone	benthic zone	profundal zone
metalimnion	epilimnion	epilimnion
Shigella	Vibrio	E.coli
nitrogen fixation	all of these	nitrogen fixation
Protozoa	None of the above	Cyanobacteria
thermoacidophiles	cyanobacteria	cyanobacteria
Sea weed	Grass	Phytoplankton

are highly susceptible	produce a blue-green pigment	make their own food
approximately equal to half		greater
Coagulant	Flocculants	Disinfectant
17%	77%	71%
.Neustonic	Algal bloom	Zoogloal film
Paleic zone	Profoundal zone	Littoral zone
Protozoa	Algal bloom	Bacteria
Aquarium	Aquifers	Aquifers
Geology	Aqualogy	Hydrogeology
<i>Rhizobium</i>	<i>Nostoc</i>	<i>Rhizobium</i>
Combined sewers	Solid sewers	Solid sewers
30-40	60-80	30-40
4.5-5.5	5.5-6.5	6.5-7.5
20 days	200 hours	20 minutes
aerotolerant anaerobe	alkalophile	osmotolerant
below 0°C	above 38°C	between 16-38°C
chemotrophs	organotrophs	organotrophs
E. coli	virus	Yeast and moulds
uses oxygen when present	requires less oxygen than is present in air	uses oxygen when present
facultative	microaerophile	microaerophile
succinate	light	ammonia
too little water in the field	the toxin produced by the bacterium	dehydration and loss of nutrients











## Unit II

### **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 oceanic zone 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 bacteria form 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 the pelagic zone, the photic zone may be called the limnetic zone and the aphotic zone may be called the profundal 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. They can be further divided into four zones: vegetation zone, open water, bottom mud and surface film. The size and depth of ponds often varies greatly with the time of year; many ponds are produced by spring flooding from rivers. Food webs are 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 for amphibian breeding.<sup>[7]</sup> 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.

## **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 into upland 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. Environmental threats 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. 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. The Norfolk Broadlands in England

illustrate similar decline with pollution and invasive species. 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 ecosystems include substrate type, water depth, nutrient levels, temperature, salinity, and flow.<sup>[7][10]</sup> 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.<sup>[16]</sup> 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 (*Calymene 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 own biomass.<sup>[16]</sup> Euryhaline organisms are salt tolerant and can survive in marine ecosystems, while stenohaline or salt intolerant species can only live in freshwater environments.

## **Sanitary Analysis of Waters**

Monitoring and detection of indicator and disease-causing microorganisms 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.

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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.
5. 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.
6. 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 indicator 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 completed 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 derived 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 become 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 conditions. An example of a resuscitation step is the use of a 2 hour incubation 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 especially 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 coliforms are now available. The presence-absence test (P-A test) can be used for coliforms. This is a modification of the MPN procedure, 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 bromocresol 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- $\beta$ -D-galactopyranoside (ONPG) and 4-methylumbelliferyl- $\beta$ -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 previous standards is the requirement to have waters free of coliforms and fecal coliforms. If coliforms are present, fecal coliforms 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 microbiological quality of drinking waters has been developed, including standards for coliforms, viruses, and *Giardia*. If unfiltered 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.

## III B. Sc Microbiology –Environmental Microbiology Unit III

### LECTURE PLAN

LECTURE PLAN - UNIT -3			
S. no	Lecture duration(Hr)	Topics covered	Supporting materials
1	1	Carbon cycle-Microbial degradation of cellulose, hemicelluloses	R1-1222-1223
2	1	Carbon cycle-Lignin and chitin	T1-413-415
3	1	Nitrogen cycle-Nitrogen fixation, ammonification	T1- 427-431
4	1	Nitrification, denitrification & nitrate reduction	R2-431-450
5	1	Phosphorous cycle, phosphate immobilization & solubilization	T1- 447-450
6	1	Sulphur cycle	T1 435-446
7	1	Iron cycle	T1-450-454
8	1	Manganese cycle	T1 454-456
<b>Textbooks :</b>		T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4 <sup>th</sup> Edition.	
<b>Reference books:</b>		R1-Fudamentals of ecology– Odum Ep & Barret GW. 5 <sup>th</sup> Edition R2- Encyclopedia of Microbiology. 2 <sup>nd</sup> Edition	
<b>Website:</b>		-	
<b>Journals:</b>		-	

### Unit III

#### Question

#### Option 1

#### Option 2

_____ are mixed cultures of naturally occurring beneficial microorganisms	Enhanced microorganisms	Efficient microorganisms
_____ is a process of bioremediation used to improve quality of water	Bioaccumulation	Biostimulation and bioaugmentation
_____ bioremediation involves treating the contaminated site	<i>Ex situ</i>	<i>In situ</i>
_____ is a method of composting by piling biodegradable waste	Windrow method	Window method
Addition of archaea or bacterial cultures to speed up the rate of bioremediation	Bioaugmentation	Bioriching
An engineered device or system that supports a biologically active culture	Incubator	Shake flask
Bacteria used in bioleaching	<i>Vibrio cholerae</i>	<i>Salmonella typhi</i>
Biostimulation is the process of	Organic farming	Enhancing growth of microorganisms
Common metal contaminants of sea water	Magnesium	Aluminium
Fungi that have the ability to degrade an extremely diverse range of organic materials	<i>Aspergillus niger</i>	<i>Mucor</i>
Intracellular accumulation of environmental pollutants such as heavy metals	Bioriching	Biobleaching
Oldest form of waste treatment/disposal	Burning	Shredding
Process of supplying oxygen in situ to microbes in contaminated soil	Bioenhancing	Bioventing
Technique that involves the use of organisms to remove or neutralize pollutants	Bioremediation	Disposal
The use of living microorganism to degrade environmental pollutants	microbioremediation	nanoremediation
The process of converting environmental pollutants into harmless substances	bioextraction	microbial extraction
<i>Ex situ</i> bioremediation involves the	Degradation of pollutants	Removal of pollutants
Which of the following is not an application of bioremediation	Treating oil spills by bioremediation	Sewage treatment by bioremediation
Which of the following is not true with respect to the advantages of bioremediation	Inexpensive	Cost effective
Bioremediation is the process of removing contaminants or pollutants from the environment	Chemical	Abiotic
The addition of known active microbes to soil or water with the aim of bioremediation	Biodegradation	Bioremediation
Which microorganisms are most commonly used bacteria for bioremediation	<i>Bacillus</i>	<i>Klebsiella</i>
Advanced treatment is generally used to treat waste water to	remove coarse solids	remove settleable solids
The concept of putting microbes to help clean up the environment is	pasteurization	bioremediation
Treatment of municipal water supplies is based upon	coagulation, filtration, chlorination	coagulation, filtration, chlorination
which of the following is generally not referred to the sewerage system	Sanitary sewers	Storm sewers
The magnitude of BOD of wastewater is related to	bacterial count	amount of organic material
<i>Chlorella pyrenoidosa</i> is usually found in	Activated sludge process	Sludge compost
Schmutzdecke is a hypogeal biological layer formed on surface of activated sludge	Fungi	Bacteria
Sludge conditioning is accomplished by which of the following	Thickening	Elutriation
A dense bacterial population caught in a tangled web of fibers is called	bio-disc	coagulation
The filtering medium of the tank becomes coated with a microbial film	Biofilm	Zoogloeal film
The most commonly efficient substrate used as a carbon source in wastewater treatment	Methanol	Oxygen
The optimum rate of relative humidity for the survival of the microorganisms	40-80%	60-80%
Which of the following is not an aerobic process?	Activated sludge process	Sludge digestion
Zoogloeal film formed in the trickling filter consists of	Bacteria	Algae
Biogas production is	a temperature independent process	a temperature-dependent process



Which of the following method is used for removal of suspended solids?	Filtration	Purification
Which of the following promotes the biological transformation of organic matter?	Primary	Secondary
Inorganic nutrients are removed by biological means refer as wastewater treatment.	Primary	Secondary
In industrial processing plants, which of the following is the primary method of waste treatment?	Removal of microbes	Removal of organic matter
Along with inorganic and organic nutrients which of the following is a common pollutant?	Heavy and trace metals	Lignocellulosic materials
Algal bloom results in _____	Global warming	Salination
Copper is used in water treatment as a _____	Disinfectant	Indicator
Formation of _____ is crucial step in anaerobic digestion	Hydrogen	Carbondioxide
Most abundant pollutant in the atmosphere among hydrocarbons is _____	methane	propane
Which of the following bacterium is called as the superbug that causes food poisoning?	Bacillus subtilis	Pseudomonas putida
Which of the following is not employed as an oxidation method?	Trickling filters	Oxidation ponds
What is an anaerobic digester?	Digests food	Microbe that eats hazardous waste
The biogas production process takes place at the temperature _____	lesser than 25°C	25-40°C
Activated sludge contains large number of _____	bacteria	yeasts and molds
Trickling filter comes under _____	primary treatment	secondary treatment
Removal of solids is generally considered as a _____	Primary treatment	Secondary treatment
Microaerophilic bacteria are those which require _____	21 % oxygen for growth	low levels of oxygen
An organism is completely dependent on atmospheric O <sub>2</sub> for growth	obligate aerobe	osmotolerant
Biomass _____	provides about 50% of the world's energy	consists largely of woody material
The acetate-utilizing methanogens are responsible for _____	20% of methane production	50% of methane production











**Y (KARPAGAM ACADEMY OF HIGHER EDUCATION)**

**DEPARTMENT OF MICROBIOLOGY**

**MENTAL MICROBIOLOGY 15MBU503**

**III BSc Microbiology**

<b>Option 3</b>	<b>Option 4</b>	<b>Answer</b>
Effective microorganism	Good microorganisms	Effective microorganisms
Bioventing	Biodegradation	Biostimulation and bioleaching
<i>In vivo</i>	<i>In vitro</i>	<i>In situ</i>
Landfilling	In-vessel composting	Windrow method
Biobleaching	Biomediation	Bioaugmentation
Biofilter	Bioreactor	Bioreactor
<i>Acidithiobacillus ferrooxidans</i>	<i>Lactobacillus casei</i>	<i>Acidithiobacillus ferrooxidans</i>
Metal ore leaching	Isolation & Screening	Enhancing growth conditions of
Copper	Zinc	copper
<i>Fusarium</i>	<i>Phanaerochaete chrysosporium</i>	<i>Phanaerochaete chrysosporium</i>
Biomediation	Bioaccumulation	Bioaccumulation
Vermicomposting	Landfill	Landfill
Biomediation	Bioriching	Bioventing
Sewage treatment	Waste removal	Bioremediation
bioremediation	all of these	bioremediation
biofiltration	bioleaching	bioleaching
Degradation of pollutants	bioremediation	Removal of pollutants and collection
Burning of petroleum products	Removal of grease by bacteria that digest oil	Burning of petroleum products followed by
Contaminants are not completely removed	Environment friendly and natural process	Contaminants are not completely removed
biological	Homeostatic	Biological
Bioaugmentation	Bioaugmentation	Biodegradation
Staphylococcus	Streptococcus	Bacillus
reduce BOD	remove additional objectionable substances	Additional objectionable substances
fermentation	biologicals	bioremediation
filtration, coagulation, flocculation	coagulation, chlorination, filtration	coagulation, filtration,
Combined sewers	Solid sewers	Solid sewers
amount of inorganic nitrogen	all of the above	amount of organic material
Trickling filter	Oxidation pond	Activated sludge process
Protozoa	Algae	Bacteria
Chemical conditioning	Diluting with water	Thickening
the membrane filter	a biofilm	a biofilm
Neustonic	Algal bloom	Zoogeocal film
Glucose	Sucrose	Methanol
50-80%	30-80%	40-80%
Trickling filter	Oxidation pond	Trickling filter
Protozoa	Algal bloom	Bacteria
an oxygen dependent process	none of the above	a temperature-dependent process

sedimentation	settlement	Sedimentation
Tertiary	Quaternary	Secondary
Tertiary	Quaternary	Tertiary
Removal of solids	Removal of liquids	Removal of organics
Suspended matters	Floating materials	Heavy and trace metals
Eutrophication	Biomagnification	Eutrophication
Coagulant	Flocculants	Disinfectant
Water	Acetate	Acetate
butane	benzpyrene	methane
Pseudomonas denitr	Bacillus denitrificans	Pseudomonas putida
Contact aerators	All of these	All of these
Method to convert agr	All of the above	Method to convert agri
45-60°C	all of these	all of these
protozoa	all of these	all of these
tertiary treatment	none of these	secondary treatment
Tertiary treatment	quaternary treatment	Primary treatment
oxygen for activation	50 % oxygen for growth	low levels of oxygen for
acidophile	facultative anaerobe	obligate aerobe
is unlikely to be a maj	offers the consumer high quality energy with	consists largely of wood
70% of methane produ	85% of methane produced in a biogas reactor	70% of methane produ













microbes

ction at a place to facilitate microbial degradation  
atng on the surface of the sea  
destroyed

## UNIT III

### 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:

Medical centres  
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:

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## **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 recovery)

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

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## 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.

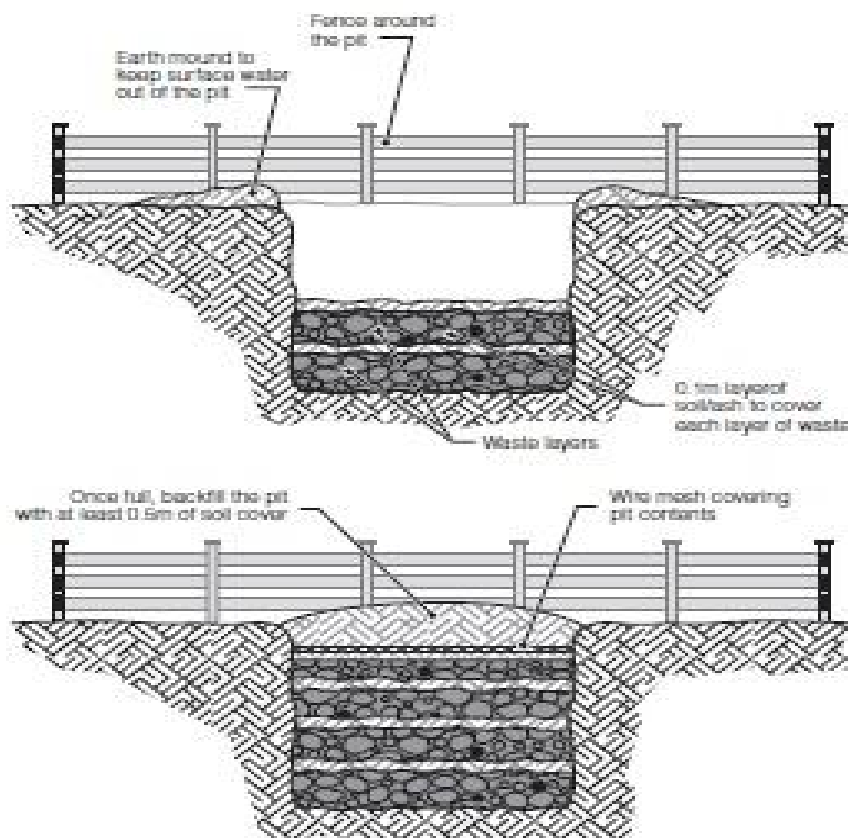
### Communal pit disposal

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

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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 promotion programme.

**Constraints:**

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

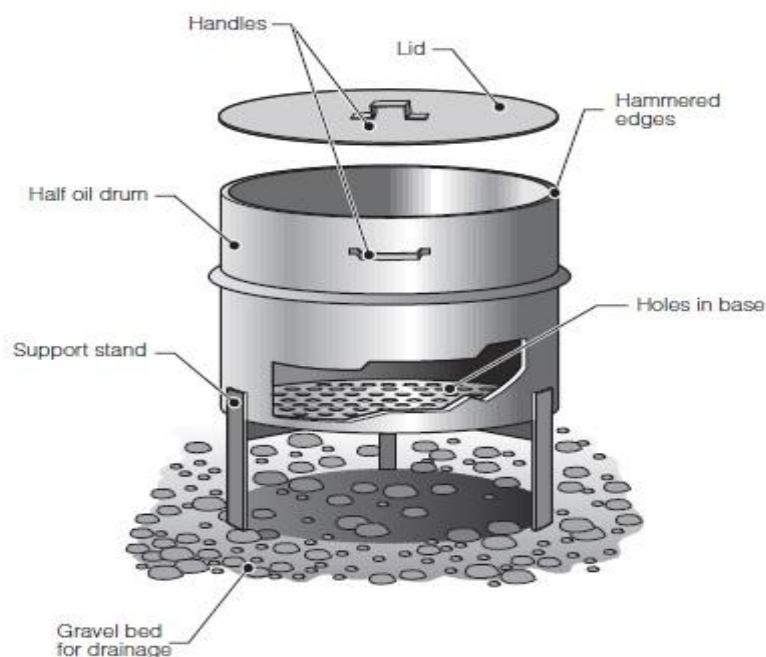
**Communal bins**

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

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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**

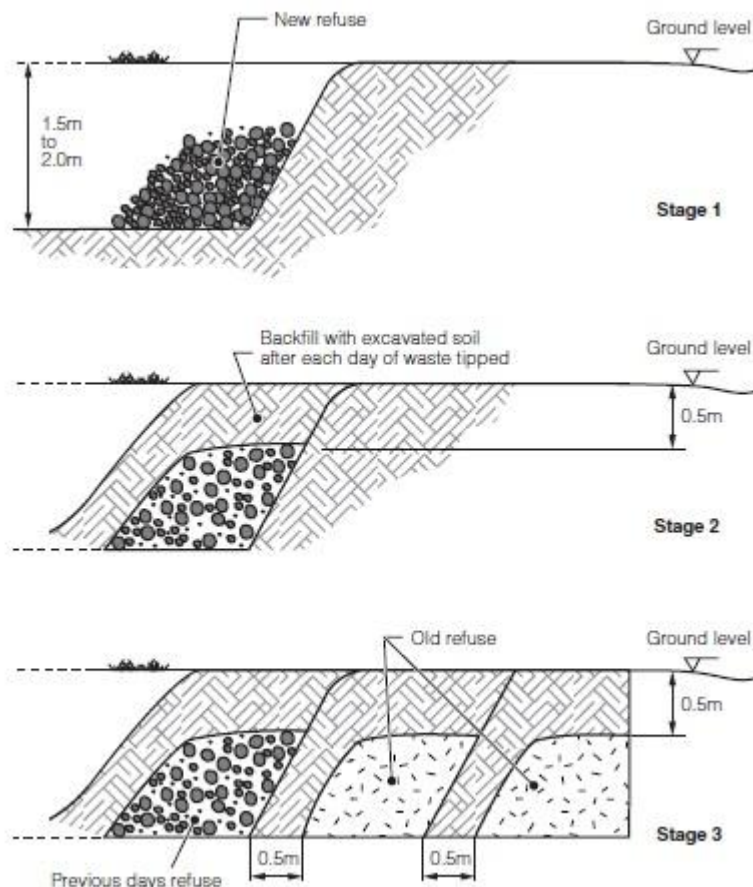
The technology choices outlined below are general options for the final disposal of waste off- site.

### **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 appropriate 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

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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 °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  $PO_4$  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.

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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  $k=0.23 \text{ d}^{-1}$  at  $20^\circ\text{C}$ ) the BOD<sub>5</sub> 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 and 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.

**III B. Sc Microbiology –Environmental Microbiology**  
**Unit IV**

**LECTURE PLAN**

LECTURE PLAN - UNIT -4			
<b>S. no</b>	<b>Lecture duration(Hr)</b>	<b>Topics covered</b>	<b>Supporting materials</b>
1	1	Solid waste management sources and types of solid waste	R1-
2	1	Methods of solid waste disposal-composting sanitary landfill	T1-413-415
3	1	Liquid waste management composition and strength of sewage BOD	W1- 2-4
4	1	Liquid waste management COD	W1-5
5	1	Primary sewage treatment	R2 602
6	1	Secondary sewage treatment oxidation ponds, trickling filter	R2 602-610
7	1	Secondary sewage treatment-activated sludge process & septic tank	R2-608-610
8	1	Tertiary sewage treatment	R2- 611-614
<b>Textbooks :</b>		T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4 <sup>th</sup> Edition.	
<b>Reference books:</b>		R1-Fudamentals of ecology– Odum Ep & Barret GW. 5 <sup>th</sup> Edition R2- Microbiology- Pelzar, Chan, Kries 5 <sup>th</sup> Edition	
<b>Website:</b>		Nptel.ac.in/courses	
<b>Journals:</b>		-	

## Unit IV

### Question

### Option 1

### Option 2

Which gas has greatest effect on global warming?	Ethane	Methane
A high biological oxygen demand	Water is pure	absence of microbia
The most important energy-yielding reaction for an aerobic organism	Glycolysis	EMP
The proteinaceous compound are converted to ammonia by	Putrifaction bacteria	Ammonification bacteria
Most bacteria require vitamins as	Growth Factors	Sources of energy
Which of these is a trace element for bacteria?	Mg+2	Na+
Nitrites are oxidized to nitrates by a microorganism	Nitrosomonas	Nitrosococcus
The main product of glycolysis under anaerobic conditions is	Pyruvate	Lactate
Antagonism “ is seen in	Lag phase	Plasmids
_____ is the major raw material for bio gas.	Cow dung	Mud
Energy transformation through the food chain is _____	Inefficient	Proposed
Oil spills are a source of pollution for _____	Land and Water	Water
The first Environment Law in India was enacted in _____	1950	1947
Bio gas generation is mainly based on the principles of	fermentation	degradation
The green house gases, otherwise called radioactively active gas	CO <sub>2</sub>	O <sub>2</sub>
Which of the following is an organic gas?	Hydrocarbons	Aldehydes
Which of the following microbe is widely used in the removal of	Trichoderma sp	Aspergillus niger
_____ normally absorb CO <sub>2</sub> from atmosphere	Ocean	River
_____ are mixed cultures of naturally occurring beneficial microorganisms	Enhanced microorganisms	Efficient microorganisms
Energy resources derived from natural organic materials are called---	geothermal energy sources	fossil fuels
A permeable rock that contains hydrocarbon fluids and gases is called	oil trap	source bed
Solar energy stored in material such as wood, grain, sugar and municipal waste	fossil fuels	biomass
What type of energy is derived from heated groundwater?	solar	geothermal
_____ is a mixture of 50 - 90% of methane	Natural gas	Air
Gasification of biomass is one of the means to harvest energy through	hydro-chemical	thermo-chemical
Gobar gas is obtained from _____	manure	cow dung
_____ is derived recently from living organisms and their metabolic products	Biofuel	Biomass
Biogas is composed of _____	methane, carbon dioxide	Carbon dioxide, nitrogen
_____ sewage is the waste water from Kitchens, bathroom and laboratories	Domestic	Commercial
_____ is a key ingredient in organic farming	Compost	Urea
_____ involves the removal of the contaminated material	<i>In vitro</i>	<i>In vivo</i>
_____ is a marine bacterium currently thought to be the	<i>Volvox globator</i>	<i>Zooglea ramigera</i>
_____ is an underground layer of water-bearing permeable rock	Bore well	Ground well
_____ is a method of composting by piling biodegradable waste	Windrow method	Window method
A bacterium often used in hydrocarbon degradation	<i>Pseudomonas putida</i>	<i>Alcaligenes</i>
Cows can digest straw because they contain	Cellulose hydrolyzing bacteria	Protein hydrolyzing bacteria

The microbial ecosystem of soil includes	biotic components of soil	abiotic components of soil
Which are the main sources of bio fertilisers?	Cyanobacteria	Bacillus
The groups of bacteria which have the ability to fix nitrogen are	symbiotic	nonsymbiotic
Which of the following is correct?	Mycorrhizae are fungi	The fungi aid in translocation of nutrients
The groups of symbiotic bacteria, which have the ability to fix nitrogen	derive their food and minerals from the host	grow together for a mutual benefit
The diagnostic enzyme for denitrification is	nitrate reductase	nitrate oxidase
Nitrogen fixation by the microorganisms can be detected by adopting	demonstrating growth in nitrogen-free medium	cultivating the microorganism in the presence of nitrogen
The phenomenon of commensalism refers to a relationship between	One species of a pair benefits	both the species of a pair
A heterocyst is	a type of spore	a terminally differentiated cell
_____ play a key role in the transformation of rock to soil.	Cyanobacteria	Pectin decomposing bacteria
The organisms responsible for the characteristic musty or earth odor	Clostridium	Streptomyces
Which of the following soil microorganism is involved in the reduction of sulfur	Thiobacillus thiooxidans	Desulfotomaculum
Composting is one of the oldest forms of disposal of waste. It is the	Ants	Bugs
Which is not a form of biomass energy?	Incineration of solid waste	Composting to produce biogas
The use of microbes to break down synthetic waste products such as	bioinformatics	bioplastics
Technique of SCP is introduced by	Gregor Mendel	Louis Pasteur
By using single-cell protein, amount of protein that can be produced	20 tons	30 tons
Substrate used by microorganisms to produce single-cell proteins include	methane gas	industrial wastes
By using single-cell protein, amount of protein produced by 50 kgs of	180 tons	100 tons
Protein content which is extracted from mixed or pure cultures of yeast	triple cell protein	single cell protein













**Y (KARPAGAM ACADEMY OF HIGHER EDUCATION)**  
**DEPARTMENT OF MICROBIOLOGY**  
**MENTAL MICROBIOLOGY 15MBU503**  
 III BSc Microbiology

<b>Option 3</b>	<b>Option 4</b>	<b>Answer</b>
CO <sub>2</sub>	Hydrogen	Methane
Low level of microb	High level of microbial pollution	High level of microbial pollution
KDPG	Both b and c	Both b and c
Nitrification bacteria	Denitrifying bacteria	Ammonification bacteria
Sources of carbon	Sources of electron donors	Growth Factors
Ca <sup>2+</sup>	Mn <sup>2+</sup>	Na <sup>+</sup>
Nitrobacter	Azotobacter	Nitrobacter
None of these	Both a and b	Pyruvate
Log phase	None of these	None of these
Plant leaves	Grass	Cow dung
Regular	Comfortable	Inefficient
Land and Air	Air and Noise	Land and Water
1972	1982	1950
purification	sedimentation	fermentation
NO <sub>2</sub>	NO	CO <sub>2</sub>
Ketones	Ammonia	Ammonia
Pseudomonas putida	Bacillus	Aspergillus niger
Lake	Pond	Ocean
Effective microorgan	Good microorganisms	Effective microorganisms
biomass	all of these	fossil fuels
oil reservoir	none of these	oil reservoir
geothermal energy	natural gas	biomass
hydroelectric	nuclear	geothermal
Water	Bio diesel	Natural gas
chemical-gaseous	hydro-thermal	thermo-chemical
crop residues	fossil	cow dung
Fossil fuel	Gobar gas	biomass
ethane, carbon dioxide	methane, carbon dioxide, nitrogen and sulphur	methane, carbon dioxide, nitrogen
Industry	Inplant	Domestic
Pesticide	Fungicide	Compost
<i>Ex situ</i>	<i>In situ</i>	<i>Ex situ</i>
<i>Chlorella pyrenoidosa</i>	<i>Alcanivorax borkumensis</i>	<i>Alcanivorax borkumensis</i>
Aquarium	Aquifers	Aquifers
Landfilling	In-vessel composting	Windrow method
<i>Peptococcus</i>	<i>Sulfobacter</i>	<i>Pseudomonas putida</i>
Lipid hydrolyzing mic	Amino acid degrading bacteria	Cellulose hydrolyzing microorganism

biotic and abiotic components of soil

Streptococcus      None of these

both (a) and (b)      none of these

The increased nutrient availability

these bacteria are from all of the above

nitro oxidoreductase      none of these

measuring  $^{15}\text{N}_2$  by mass spectrometry

One species of a pair is a pathogen

the progenitor of cyanobacteria is a cell that carries out oxygenic photosynthesis

Nitrifying bacteria      De-nitrifying bacteria

algae      virus

Rhodospirillum      Rhodomicrobium

Snakes      worms

Ethanol and methanol      Photovoltaic production of hydrogen

biotechnology      bioremediation

Professor Scrimshaw      Ian Wilmot

40 tons      50 tons

agricultural wastes      all of above

300 tons      250 tons

double cell protein      tetra cell protein

biotic and abiotic components of soil

Cyanobacteria

both a and b

All of the above

all of the above

nitrate reductase

all of the above

One species of a pair benefits

a terminally differentiated cell that

Cyanobacteria

Streptomyces

Desulfotomaculum

worms

Photovoltaic production of hydrogen

bioremediation

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20 tons

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and hydrogen

isms

oil

: fixes nitrogen

en

## UNIT IV

### Sewage Characteristics

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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 and 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.

## **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:

- Medical centres
- 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

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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 recovery)

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.

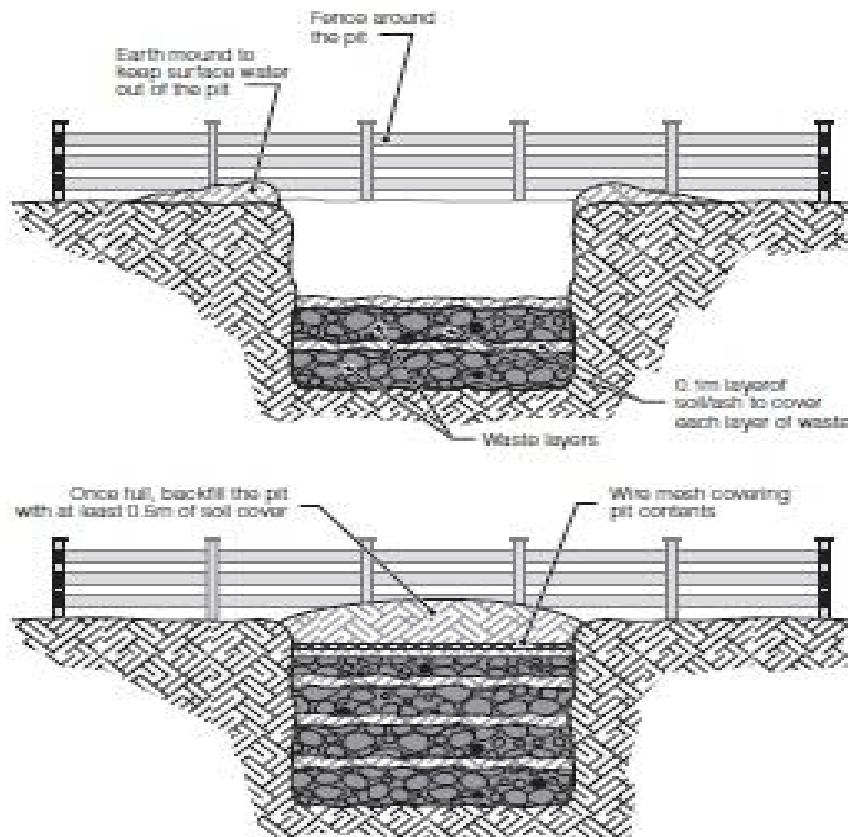
## **Communal pit disposal**

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 promotion programme.

**Constraints:**

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

## Communal bins

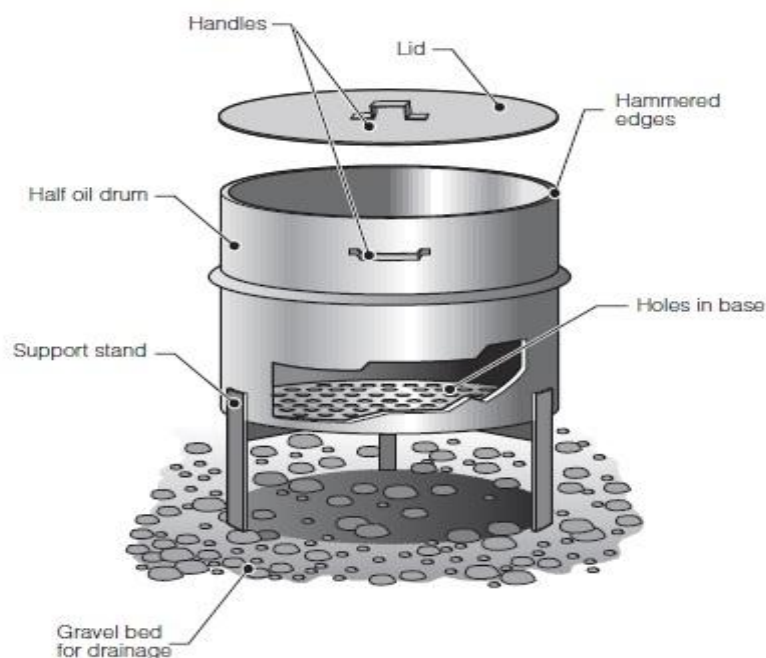
Communal bins or containers are designed to collect waste where it will not be dispersed by wind or animals, and where it can easily be removed for transportation and disposal. Plastic containers are generally

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**

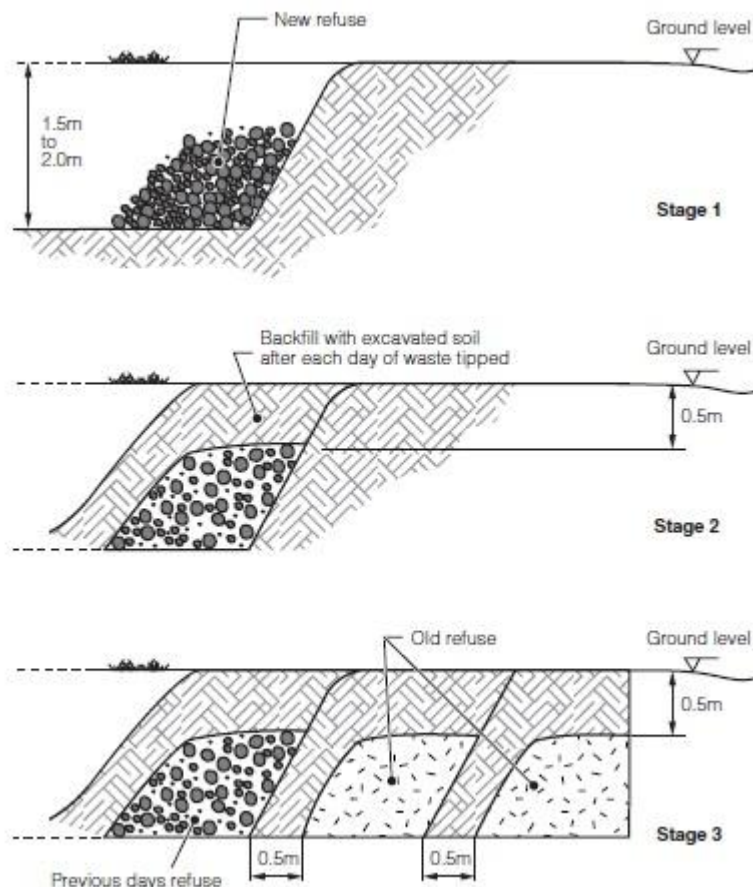
The technology choices outlined below are general options for the final disposal of waste off- site.

### **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 appropriate 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

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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.



**III B. Sc Microbiology –Environmental Microbiology  
Unit V**

**LECTURE PLAN**

LECTURE PLAN - UNIT -5			
<b>S. no</b>	<b>Lecture duration(Hr)</b>	<b>Topics covered</b>	<b>Supporting materials</b>
1	1	Principles and biodegradation of common pesticides	R1-
2	1	Organic (hydrocarbons, oil spills)	T1-413-415
3	1	Inorganic (metals) matter, biosurfactants.	W1- 2-4
4	1	Treatment and safety of drinking (potable) water	W1-5
5	1	Methods to detect potability of water samples: MPN test	R2 602
6	1	Methods to detect potability of water samples: Membrane filter technique.	R2 602-610
7	1	GMO and their impact.	R2-608-610
8	1	Tertiary sewage treatment	R2- 611-614
<b>Textbooks :</b>		T1- Microbial Ecology: Fundamentals & Applications – Atlas RM & Bartha (2000) 4 <sup>th</sup> Edition.	
<b>Reference books:</b>		R1-Fudamentals of ecology– Odum Ep & Barret GW. 5 <sup>th</sup> Edition R2- Microbiology- Pelzar, Chan, Kries 5 <sup>th</sup> Edition	
<b>Website:</b>		Nptel.ac.in/courses	
<b>Journals:</b>		-	

## Unit V

### Question

### Option 1

### Option 2

_____ are aerobic and free-living nitrogen nitrogen fixers	<i>Frankia</i> & <i>Azospirillum</i>	<i>Clostridium</i> & <i>Desulfohalobium</i>
_____ are genes encoding enzymes involved in the fixation of nitrogen	<i>nif</i>	<i>nif</i>
_____ catalyze conversion of atmospheric nitrogen to ammonia	Kinase	Hydrogenase
_____ is a typical example of symbiotic nitrogen fixation seen in	<i>Azolla-Anabaena</i>	<i>Alder-Frankia</i>
_____ recycles the H <sub>2</sub> produced during N <sub>2</sub> fixation, thereby	Reductase	Catalase
A free-living anaerobic photosynthetic bacterium	<i>Anabaena azollae</i>	<i>Clostridium thermocellum</i>
A free-living soil bacteria that is involved in nitrogen fixation	<i>Alcaligenes</i>	<i>Acetobacter</i>
Amount of ATP needed to form 2 moles of ammonia from 1 mole of N <sub>2</sub>	8	16
Apart from biological nitrogen fixation by microbes, _____	Cyclone	Thunder
Bacteria that forms root nodules in legume plants	<i>Rhizobium</i>	<i>Azotobacter</i>
Biological nitrogen fixation was discovered by	Winogradsky	Beijerinck
Chemicals produced by the Rhizobia called _____ that call for	Pod factors	Nod factors
Example of associative nitrogen fixation	<i>Legume-Rhizobium</i>	<i>Rice-Azospirillum</i>
<i>Frankia</i> is a	Bacteria	Actinomycete
Group of irregularly shaped bacteria in root nodules are called	Bacteroids	Asteroids
In biological nitrogen fixation, _____ moles of ammonia are produced	2	4
In Cyanobacteria, nitrogen fixation occurs in terminally differentiated	Cyanocysts	Nitrocysts
In root nodules, _____ bind and regulate the levels of oxygen	Teghemoglobin	Peghemoglobin
Legume plants belongs to	<i>Solanaceae</i>	<i>Rosaceae</i>
Most abundant gas in atmosphere	Nitrogen	Oxygen
Nitrogenase enzyme consists of	Iron protein	Molybdenum-iron protein
Rhizobia are attracted to _____ released by the host legume	Flavonoids	Enzymes
The enzyme nitrogenase is inhibited by	CO <sub>2</sub>	Sulfur
Which is not true about <i>Anabaena</i> and <i>Nostoc</i>	Filamentous	Nitrogen fixing
The majority of hydrogenases in prokaryotes are _____ controlled	Nickel	Copper
With associative nitrogen fixation, which one of the following genera	<i>Azotobacter</i>	<i>Escherichia</i>
The conversion of nitrogen to ammonia or nitrogenous compounds is called	Nitrogen assimilation	Nitrogen fixation
The organisms which is not symbiotic nitrogen fixing cyanobacteria	<i>Anthoceros</i>	<i>Azolla</i>
All the following are free living nitrogen fixers except _____	<i>Rhizobium</i>	<i>Azotobacter</i>
<i>Anabaena</i> is a nitrogen fixer present in the root pockets of _____	<i>Marselia</i>	<i>Salvinia</i>
Splitting of dinitrogen molecule into free nitrogen atom in biological	hydrogenase	nitrogenase
Which of the following aid plants in the acquisition of nitrogen from	Bacteria	Algae
A major plant macronutrient found in nucleic acids and proteins is _____	calcium	nitrogen
Organisms capable of converting nitrogen to nitrate are _____	yeast	bacteria
Conversion of nitrite to nitrate is carried out by _____	<i>Nitrosomonas</i>	<i>Nitrosococcus</i>
The nonsymbiotic bacteria which fix nitrogen live in the soil independently	<i>Azotobacter</i>	<i>Anabaena</i>
Which of the following is not the biofertilisers producing bacteria?	<i>Nostoc</i>	<i>Anabaena</i>

Which of the following is capable of oxidizing sulfur to sulfates? Thiobacillus thiooxidans Desulfotomaculum

Nitrifying bacteria can not be isolated directly by the usual technique slow growth medium growth

\_\_\_\_\_ play a key role in the transformation of rock in the transformation Cyanobacterium pectin decomposing bacteria

Denitrification may be distinguished as dissimilative and assimilative

All of the following are examples of negative symbiosis \_\_\_\_\_ amensalism competition

The reservoir for nitrogen is \_\_\_\_\_ the atmosphere rocks

Most soil protozoa are flagellates or amoebas, having their dominant mode of feeding Ingestion of bacteria ingestion of mold

Nitrifying bacteria can not be isolated directly by the usual technique medium growth fast growth

The transformation of nitrates to gaseous nitrogen is accomplished by nitrification denitrification

Nitrogen fixation refers to the direct conversion of atmospheric nitrogen into ammonia glucose

The diagnostic enzyme for denitrification is \_\_\_\_\_ nitrate reductase nitrate oxidase

An example of a symbiotic nitrogen fixer is \_\_\_\_\_ Azotobacter Beijerinckia

In the process of nitrogen fixation, which of the following microorganisms are involved? Non symbiotic microorganisms Symbiotic microorganisms

The physical structure of soil is improved by the accumulation of \_\_\_\_\_ mold mycelium minerals

The conversion of molecular nitrogen into ammonia is known as \_\_\_\_\_ nitrification denitrification

Some microorganisms have the ability to increase the nitrogen content of soil Nitrogen fixation denitrification

Which of the following soil microorganism is involved in the reduction of sulfur? Thiobacillus thiooxidans Desulfotomaculum

Which of the following fungi on infecting crop roots can improve the growth of the plant? Saccharomyces cerevisiae VA Mycorrhiza

Syntrophism involves exchange of nutrients between plants and animals

Assimilative denitrification is done by \_\_\_\_\_ Plants animals

The diagnostic enzyme for nitrogen-fixing organisms is nitrogenase nitrate reductase

Which of the following is the type of endosymbiosis Commensalism cooperation

Azolla is used as biofertilizer as it has \_\_\_\_\_ Rhizobium Cyanobacteria

In 1888, a Dutch microbiologist Beijerinck succeeded in isolating Bradyrhizobium japonicum Rhizobium leguminosarum

Ammonia produced in the bacterioid needs to be transported to the lipid membrane periplasmic membrane













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**DEPARTMENT OF MICROBIOLOGY**  
**MENTAL MICROBIOLOGY 15MBU503**  
 III BSc Microbiology

<b>Option 3</b>	<b>Option 4</b>	<b>Answer</b>
<i>Beijerinckia</i> & <i>Klebsif</i>	<i>Rhizobium</i> & <i>Anabaena</i>	<i>Beijerinckia</i> & <i>nif</i>
Nitrogenase	Phosphatase	Nitrogenase
Legume- <i>Rhizobium</i>	Higher plants- <i>Mycorrhizae</i>	<i>Azolla-Anabaena</i>
Nitrogenase	Hydrogenase	Hydrogenase
<i>Rhodospirillum rubrum</i>	<i>Klebsiella pneumoniae</i>	<i>Rhodospirillum rubrum</i>
<i>Pseudomonas</i>	<i>Azotobacter</i>	<i>Azotobacter</i>
32	64	16
Raining	Lightning	Lightning
<i>Azospirillum</i>	Cyanobacteria	<i>Rhizobium</i>
Pasteur	Koch	Beijerinck
Sod factors	Mod factors	Nod factors
Higher plants-Mycor	<i>Azolla-Anabaena</i>	<i>Rice-Azospirillum</i>
Fungi	Algae	Actinomycete
Misteroids	Histeroids	Bacteroids
6	8	2
Heterocysts	Homocysts	Heterocysts
Leghemoglobin	Hemoglobin	Leghemoglobin
<i>Astraceae</i>	<i>Fabaceae</i>	<i>Fabaceae</i>
Carbon dioxide	Hydrogen	Nitrogen
Iron protein and a m	Hemoglobin	Iron protein and a molybdenum-
Toxins	Chemicals	Flavonoids
Hydrogen	Oxygen	Oxygen
Cyanobacteria	Symbiotic	Symbiotic
Molybdenum	Sulfur	Nickel
<i>Rhizobium</i>	<i>Anabena</i>	<i>Azotobacter</i>
Denitrification	Nitrification	Nitrogen fixation
<i>Cycas</i>	<i>Gnetum</i>	<i>Gnetum</i>
<i>Rhodospirillum</i>	<i>Clostridium</i>	<i>Rhizobium</i>
<i>Pistia</i>	<i>Azolla</i>	<i>Azolla</i>
dinitrogenase	nitrate reductase	nitrogenase
Nematodes	Moulds	Bacteria
sulphur	iron	nitrogen
roundworms	moulds	bacteria
<i>Nitrobacter</i>	<i>Clostridium</i>	<i>Nitrobacter</i>
<i>Rhizobium</i>	<i>Azolla</i>	<i>Azotobacter</i>
Both (a) and (b)	<i>Clostridium</i>	<i>Clostridium</i>

Rhodospirillum	Rhodomicrobium
fast growth	no growth
denitrifying bacteria	
Partially dissimilative	Partially assimilative
commensalism	parasitism
ammonia	nitrate
ingestion of fungi	ingestion virus
Good growth	slow growth
nitrogen fixation	ammonification
ATP	Nitrate
nitro oxidoreductase	reductase
Clostridium	Rhizobium
Non symbiotic and sy	Symbiosis
water	metals
nitrogen fixation	ammonification
nitrification	ammonification
Rhodospirillum	Rhodomicrobium
Candida torulopsis	Aspergillus niger
no exchange of nutri	no exchange of nutrients among species
virus	protozoans
nitrate oxidase	dehydrogenase
mutualism	predation
Mycorrhiza	Large quantity of humus
Sinorhizobium meli	Both a and b
symbiosome membr	plasma membrane

Thiobacillus thiooxidans  
 slow growth  
 Cyanobacteria  
 Dissimilative and assimilative  
 competition  
 the atmosphere  
 ingestion of bacteria  
 slow growth  
 Denitrification  
 ammonia  
 nitrate reductase  
 Rhizobium  
 Non symbiotic and symbiotic micr  
 Mold mycelium  
 nitrogen fixation  
 nitrogen fixation  
 Desulfotomaculum  
 VA Mycorrhiza  
 exchange of nutrients between two  
 plants  
 nitrogenase  
 Commensalism  
 Cyanobacteria  
 Rhizobium leguminosarum  
 symbiosome membrane











-iron protein



organisms only

species

## UNIT V

### **Positive and negative role of microbes in environment**

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 CO<sub>2</sub> 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 mutualistic 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.

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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 leguminosae). 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* and *Pseudomonas aeruginosa* towards the fungus *Aspergillus terreus*. Certain *Pseudomonas* pigments inhibit germination of *Aspergillus* spores. *Staphylococcus aureus* produces a diffusible 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 chitinous shells of dead crustaceans in the sea. Their existence, in the environment free of other microorganisms, is may be due to the production of antibiotics by them. Several species of *Streptomyces* from soil produce antibacterial and antifungal antibiotics. Most of the commercial antibiotics such as streptomycin, chloramphenicol, Terramycin and cyclohexamide have been produced from the mass culture of *Streptomyces*. 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 secrete 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.

Amensalism 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.

Exogenous nutrients are required for the germination of chlamydospores of *Fusarium*, Oospores of *Aphanomyces* and conidia of *Verticillium dahliae* in 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.

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## **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 prey 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.

### **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 bioremediation. 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.

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## 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.

<b>Pesticide</b>	<b>Target</b>
<u>Algicides</u>	<u>Algae</u>
<u>Avicides</u>	<u>Birds</u>
<u>Bactericides</u>	<u>Bacteria</u>
<u>Fungicides</u>	<u>Fungi</u>
<u>Insecticides</u>	<u>Insects</u>
<u>Miticides or Acaricides</u>	<u>Mites</u>
<u>Molluscicides</u>	<u>Snails</u>
<u>Nematicides</u>	<u>Nematodes</u>
<u>Rodenticides</u>	<u>Rodents</u>
<u>Virucides</u>	<u>Viruses</u>

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 CO<sub>2</sub> or NH<sub>3</sub> 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 *Pseudomonas 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: both are pumps that translocate herbicides or glutathione-conjugates to vacuoles.

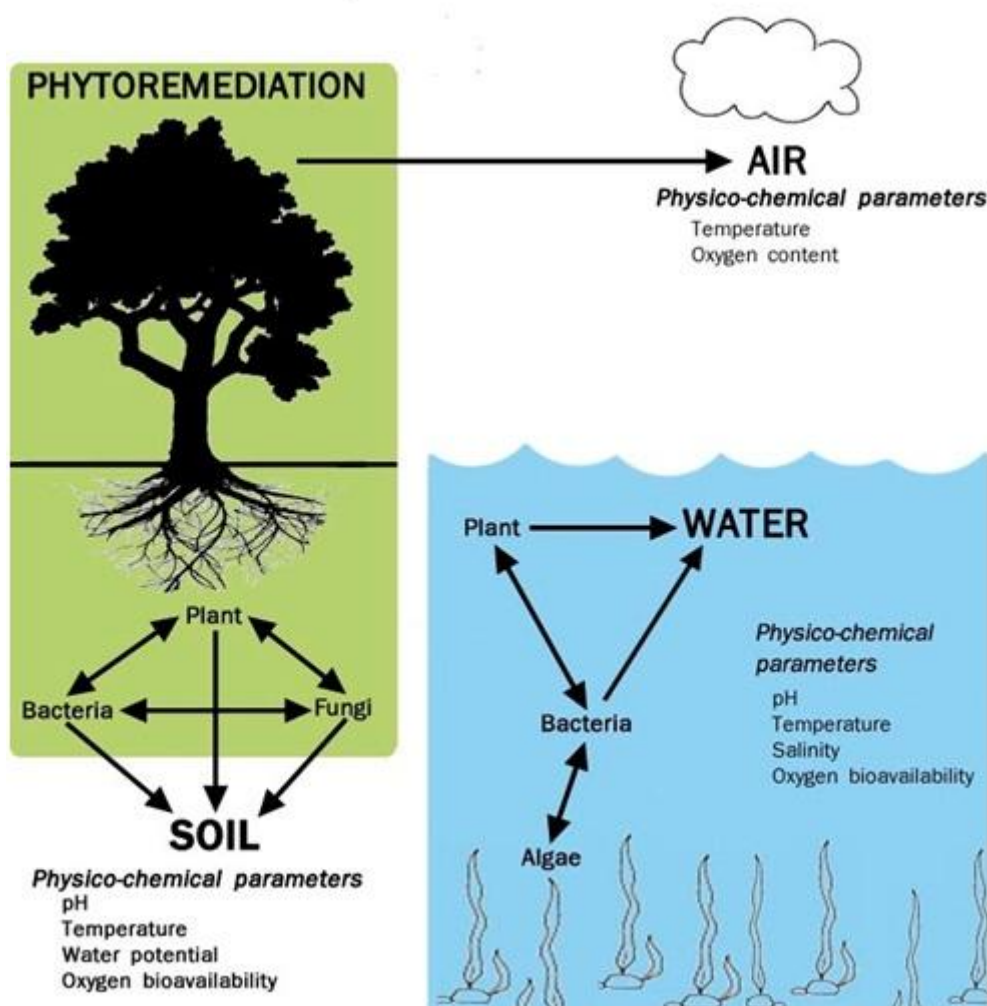
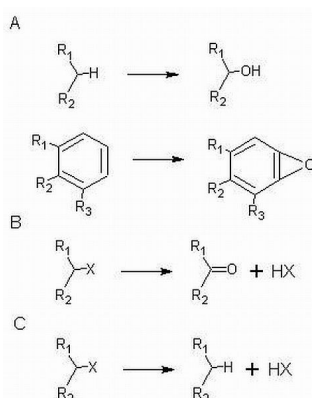


Figure 1. Factors affecting biodegradation and bioremediation in soil, water or air.

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 promiscuous 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 evolutionally 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

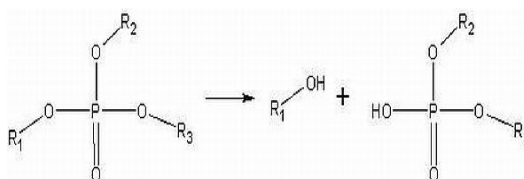


biodegradation of aromatic or alicyclic compounds and can activate toxics, i.e., CYP action on biomolecules might make them toxic or increase their toxicity.

**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 extremely sensitive to the presence of azide, and inhibitor of metalloenzymes, with the exception of lignine peroxidases from fungi. It is known that ligninolytic fungi secrete peroxidases and oxidases to degrade lignine. These enzymes are highly promiscuous.

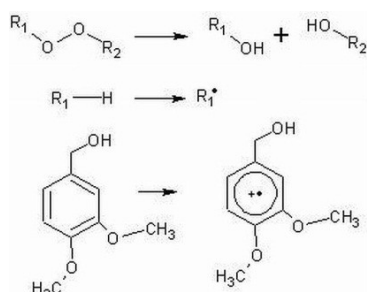


Figure 4. Scheme of reactions catalyzed by peroxidases.

### 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 electrophilic pesticides, they can also show hydrolytic and peroxidase activities. Interestingly, GST can also catalyze the de- halogenation of rings (Figure 5).

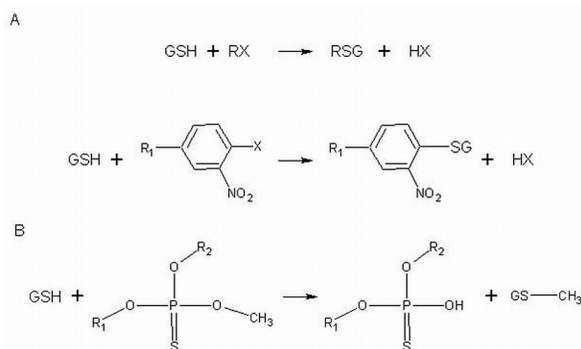


Figure 5. Scheme of reactions catalyzed by GST: A) dehalogenation, B) O-dealkylation.

### 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 .

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### **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 biodegradation 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 dichlorodiphenyltrichloroethane (DDT), metoxychlor and gamma-hexachlorocyclohexane (gamma-HCH), is affected by temperature and the ratio of desorbed pesticide. Moreover, only one 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 *Pseudomonas* 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*, *Mucor*, *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 fenitrothion 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 hydrolyze methylparathion was cloned to *Escherichia coli*. In contrast, the ability to degrade fenitrothion 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 usefulness 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,

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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 .

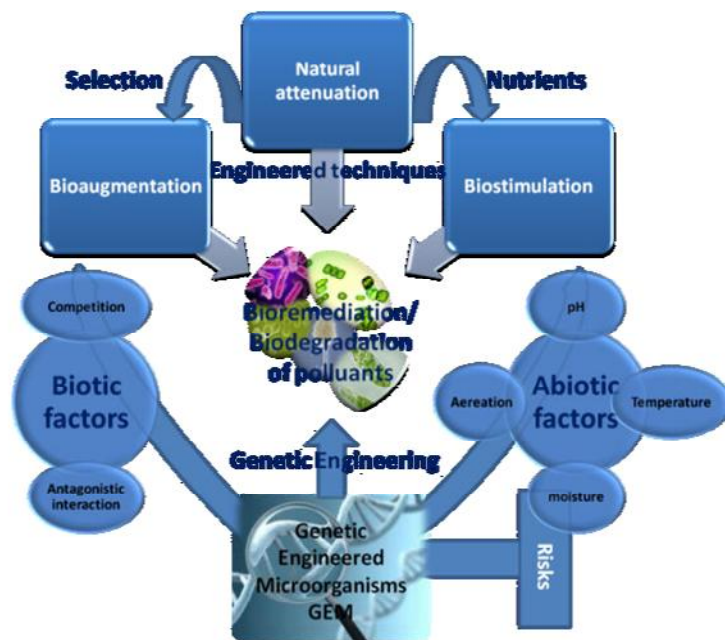


Figure 1. Bioremediation of pollutants utilizing biodegradation abilities of microorganisms include the natural attenuation, although it may be enhanced by engineered techniques, either by addition of selected microorganisms (bioaugmentation) or by biostimulation, where nutrients are added. Genetic engineering is also used to improve the biodegradation capabilities of microorganisms by GEM. Nevertheless, there are many factors affecting the efficiency of this process and risks associated to the use of GEM in the field.

### 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 ( $C_6H_6$ ) 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

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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

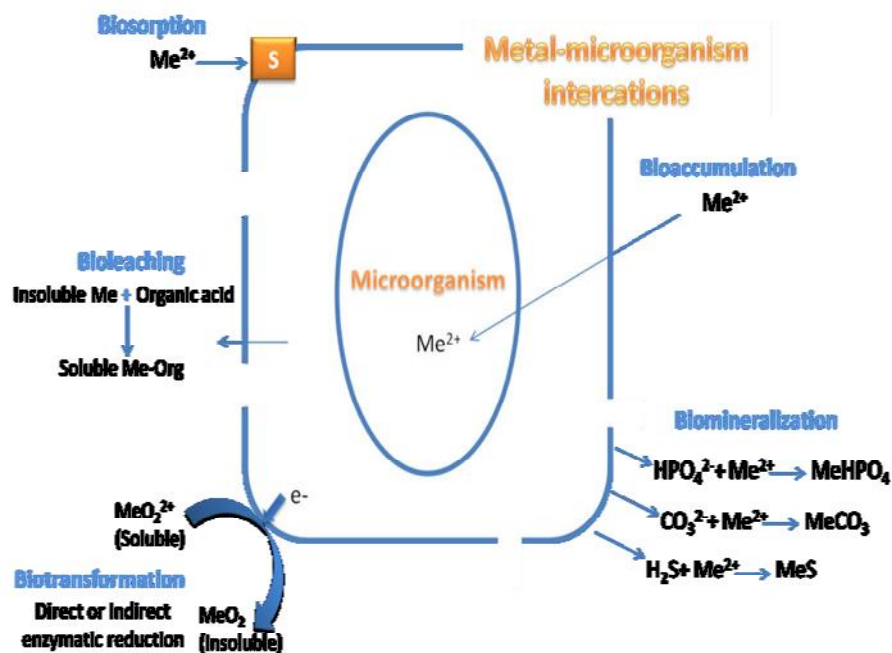


Figure 2. Microbial processes used in bioremediation

## 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

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Isolated belonged to 10 genus as follows: *Bacillus*, *Corynebacterium*, *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-dihydroxybiphenyl 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 example, 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 population density can be extrapolated to quantitative analysis of the kinetics of azo dye decoloration 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  $\text{SO}_4^{2-}$  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 enrichment 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.

### **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 engineering 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 between microorganisms. 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 microorganisms, 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

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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 recombining parts of CAM and OCT developed by conjugation could degrade camphor, octane, salicylate, and naphthalene and could grow 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-chlorobenzoate), 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, *Rhodospseudomonas palustris*, was constructed to simultaneously express mercury transport system and metallothionein for Hg<sup>2+</sup> 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 technologies 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 contaminant-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 phytochelatin and accumulate Cd<sup>2+</sup>, 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.

**Reg. No. : -----**

**[15MBU504]**

**KARPAGAM UNIVERSITY**

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

**B.Sc. DEGREE EXAMINATION, NOVEMBER 2016**

## I CIA EXAM - FIFTH SEMESTER

# MICROBIOLOGY

# ENVIRONMENTAL MICROBIOLOGY

**Time: 2.00 hours**

**Maximum: 50marks**

### PART A – (Multiple choice questions)

**(20 x 1 = 20 marks)**

- \_\_\_\_\_ virus causes whooping cough.
  - Varicella
  - Influenza
  - Bordetella pertussis
  - Rubella
- A great deal of indoor air pollution comes from\_\_\_\_\_
  - Carpets and Furniture
  - Sludge
  - Cooking
  - Carbon monoxide
- Air is composed of gases,water vapours and \_\_\_\_\_
  - Dust Particles
  - Light
  - Snowfall
  - Rainfall
- Which water is otherwise known as portable water?
  - Raw
  - Irrigation
  - Drinking
  - Surface
- The environment which has been modified by human activities is called
  - Natural
  - Urban
  - Anthropogenic
  - Modern
- The top layer of the lake is called as-----
  - Thermocline
  - Epilimnion
  - Thermonion
  - Hypolimnion
- The presence of high coliform counts in water indicates-----
  - Contamination by human wastes
  - Phosphorous Contamination
  - Decreased biological oxygen demand
  - Hydrocarbon Contamination
- 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
- Water testing relies on the detection of certain indicator organisms known as
  - acid-fast bacteria
  - bacteroids
  - coliforms
  - dinoflagellates
- 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
- \_\_\_\_\_ is an opportunistic fungal disease of human caused by inhalation of spores
  - Sporidiasis
  - Penicilliosis
  - Aspergillois
  - Candidiasis
- Cryptococcosis is caused by\_\_\_\_\_
  - C.neoformans
  - C.licheniformis
  - C.pseudopodis
  - C.parvum
- \_\_\_\_\_ is a severe respiratory disease caused by bacteria

- a) Aspergillosis
  - c) Tuberculosis
  - b) Scarlet fever
  - d) Tetanus
14. Which is not true about Anabaena and Nostoc?
- a) Filamentous
  - c) Cyanobacteria
  - b) Nitrogen fixing
  - d) Symbiotic
15. Cyanobacteria are \_\_\_\_\_
- a) Photoheterotrophs
  - c) Prototrophs
  - b) Chemotrophs
  - d) Photoautotrophs
16. Microbes in air can be enumerated by \_\_\_\_\_
- a) Settle plate method
  - c) Spread plate method
  - b) Pour plate method
  - d) Streak plate method
17. The commonest genera of fungi in indoor air are \_\_\_\_\_
- a) Saccharomyces
  - c) Rhizopus
  - b) Penicillium
  - d) Mucor
18. \_\_\_\_\_ can be a source of infectious diseases
- a) Droplets
  - c) Dust
  - b) Aerosols
  - d) Flocs
19. Air doesn't have \_\_\_\_\_ flora
- a) Indigenous
  - c) Exogenous
  - b) Endogenous
  - d) Subgenous
20. Common source of air microflora is \_\_\_\_\_
- a) Human
  - c) Industries
  - b) Soil
  - d) Vehicles

**PART C – (5 x 8 = 40 marks)**

**Answer any five questions.**

**All questions carry equal marks.**

1. Elaborate on different air sampling techniques for enumeration of microorganisms from air.
2. Describe in detail about air borne bacterial diseases.
3. Discuss about air borne viral diseases.
4. Describe in detail about air borne fungi diseases.
5. Explain in detail about marine water ecosystem .
6. Write a note on fresh water ecosystem

**KARPAGAM UNIVERSITY**  
**(Under Section 3 of UGC Act 1956)**  
**COIMBATORE – 641 021**  
**B.Sc. DEGREE EXAMINATION, AUGUST 2017**  
**FIFTH SEMESTER**  
**MICROBIOLOGY**  
**ENVIRONMENTAL MICROIOLOGY**

**Time: 2.00 hours**

**Maximum: 50marks**

**PART A – (20 x 1 = 20 marks)**

1. Most of the indicator organisms for detection of disease occurrence level in drinking water belongs to which of the following microroganisms group
  - a) Actinobacteria
  - b) Bacilli
  - c) Coliform
  - d) Firmicutes
2. Which among the following microbes is not a prime concern for deterioration of water quality in drinking water
  - a) Legionella
  - b) Shigella
  - c) Vibrio parahaemolyticus
  - d) Vibrio vulnificus
3. Which of the following test is used as preusmptive test for enumeration of coliform in water samples?
  - a) Most probable number
  - b) Heterocoliformcount
  - c) Aerobic colony count
  - d) colony forming unit
4. Which water is otherwise known as portable water?
  - a) Raw
  - b) Irrigation
  - c) Drinking
  - d) Surface
5. The presence of high coliform counts in water indicates \_\_\_\_\_
  - a) Contamination by human wastes
  - b) Phosphorous Contamination
  - c) Decreased biological oxygen demand
  - d) Hydrocarbon Contamination
6. Water testing relies on the detection of certain indicator organisms known as
  - a) acid-fast bacteria
  - b) bacteroids
  - c) coliforms
  - d) dinoflagellates
7. The best example for total coliforms is \_\_\_\_\_
  - a) E. coli
  - b) Salmonella
  - c) Shigella
  - d) Vibrio
8. Copper is used in water treatment as a
  - a) Disinfectant
  - b) Indicator
  - c) Coagulant
  - d) Flocculants
9. In the human disease cholera, what is it that actually ends up killing the victim?
  - a) Faulty carrier proteins
  - b) Dehydration and loss of nutrients
  - c) Little water in the food stream
  - d) the toxin produced by the bacterium
10. \_\_\_\_\_ are mixed cultures of naturally occurring beneficial microbes used to degrade contaminants, increase quality of soil etc
  - a) Enhanced microorganisms
  - b) Efficient microorganisms
  - c) Effective microorganisms
  - d) Good microorganisms

11. \_\_\_\_\_ is a process of bioremediation used to improve quality of underground water
  - a) Bioaccumulation
  - b) Biostimulation and bioleaching
  - c) Bioventing
  - d) Biodegradation
12. \_\_\_\_\_ bioremediation involves treating the contaminated material at the site
  - a) Ex situ
  - b) In situ
  - c) In vivo
  - d) In vitro
13. \_\_\_\_\_ is a method of composting by piling biodegradable waste in long rows
  - a) Windrow method
  - b) Window method
  - c) Land filling
  - d) In-vessel composting
14. Addition of archaea or bacterial cultures to speed up the rate of degradation of a contaminant
  - a) Bioaugmentation
  - b) Bioriching
  - c) Biobleaching
  - d) Biomediation
15. The magnitude of BOD of wastewater is related to
  - a) bacterial count
  - b) amount of organic material
  - c) Amount of inorganic material
  - d) all of the above
16. Treatment of municipal water supplies is based upon
  - a) Coagulation, filtration, chlorination
  - b) Chlorination, filtration, coagulation
  - c) Filtration, coagulation, chlorination
  - d) Coagulation, chlorination, filtration
17. Which of the following is generally not referred to the sewerage system?
  - a) Sanitary sewers
  - b) Storm sewers
  - c) Combined sewers
  - d) Solid sewers
18. Sludge conditioning is accomplished by which of the following
  - a) Thickening
  - b) Elutriation
  - c) Chemical conditioning
  - d) Diluting with water
19. A dense bacterial population caught in a tangled web of fibers sticking to a surface describes
  - a) biodisc
  - b) coagulation
  - c) The membrane filter technique
  - d) a biofilm
20. The filtering medium of the tank becomes coated with a microbial flora, the \_\_\_\_\_ film
  - a) Bio film
  - b) Zooglocal film
  - c) Neustonic
  - d) Algal bloom

**PART C – (5 x 8 = 40 marks)**

**Answer any five questions  
All questions carry equal marks.**

1. Write short notes on potability of water by MPN method.
2. Comment on major water borne diseases and their control measures.
3. Write notes on water purification.
4. Major water borne diseases and their control measures
5. Give an account on types of solid and liquid wastes.
6. Write notes on physical, chemical and biological waste treatment.

**Model**

**Reg. No. : -----**  
**[15MBU504]**

**KARPAGAM UNIVERSITY**  
**(Under Section 3 of UGC Act 1956)**  
**COIMBATORE – 641 021**  
**B.Sc. DEGREE EXAMINATION, SEPTEMBER 2017**  
**FIFTH SEMESTER**  
**MICROBIOLOGY**  
**ENVIRONMENTAL MICROBIOLOGY**

**Time: 2 hours**

**Maximum: 50 marks**

**PART A – (20 x 1 = 20 marks)**  
**(Multiple Choice Question No.1 to 20 Online Exam)**

1. Microbes in air can be enumerated by
  - a) Settle plate method
  - b) Pour plate method
  - c) Spread plate method
  - d) All above
2. \_\_\_\_\_ is a source of contamination of air borne diseases
  - a) Sneezing
  - b) Coughing
  - c) Talking
  - d) All above
3. Effective air sanitizing is done by
  - a) Gamma radiation
  - b) UV radiation
  - c) Beta radiation
  - d) Nano radiation
4. The principle involved in the air sampler Hesses tube is
  - a) Centrifugal action
  - b) Settling under gravity
  - c) Filtration
  - d) impingement
5. Average salinity of seawater
  - a) 15 ppt
  - b) 25 ppt
  - c) 35 ppt
  - d) 45 ppt
6. Microorganisms found attached to the rock surface are referred to as
  - a) Epiphyton
  - b) Epizoa
  - c) Epixylon
  - d) Epilithon
7. Profundal zone is
  - a) Open surface of water body
  - b) Sub-surface zone
  - c) Deepest zone
  - d) Side zone
8. Which of the following can be seen in marine environment?
  - a) Halophiles
  - b) Barophiles
  - c) Psychrophiles
  - d) Hydrophiles
9. Addition of archaea or bacterial cultures to speed up the rate of degradation of a contaminant
  - a) Bioaugmentation
  - b) Bioriching



**(Answer all questions)**

1. a. Assessment of air quality by solid and liquid impingement methods. Or  
b. Discuss in detail about air-borne fungal diseases.
2. a. Write short notes on potability of water by MPN method. Or  
b. Write a note on marine water ecosystem.
3. a. Give an account on types of solid and liquid wastes. Or  
b. Write notes on physical, chemical and biological waste treatment.
4. a. Write a note on bioremediation of solid wastes for fuel (ethanol and methane).Or  
b. Briefly explain about utilization of solid wastes as fertilizer (composting)
5. a. Comment on biodegradation of recalcitrant compounds. Or  
b. Give an account of bioaccumulation of metals and detoxification.