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

SCOPE This course has been designed to provide the student insights into these invaluable areas of Environmental microbiology, which play a crucial role in determining its future use and applications in environmental management. Provides detailed idea about biofertilizer production and plant disease.

OBJECTIVES To educate the students about concepts of designs of water distribution systems, sewer networks, working principles and design of various physical, chemical and biological treatment systems of water and waste water. To study about the biofertilizers, plant disease and increasing soil fertility.

UNIT – I
Aquatic environment - microbiology of water - water pollution and water borne pathogens. Bacteriological examination of water, indicator organism. Microbiology of sewage. Chemical and biochemical characteristic of sewage. methods of sewage treatment - physical screening, chemical, biological (sludge digestion; activated sludge, aerating filters, oxidation pond).

UNIT – II
Microbiology of air - Microbial contaminants of air, sources of contamination, microbial indicators of air pollution. Enumeration of bacteria in air. Air samplers and Sampling techniques. Air sanitation.

UNIT – III
Bioremediation – contaminated soil, aquifers, marine pollutants, air pollutants, stimulation of oil spills degradation. Bioremediation of air pollutants. Bioleaching – recovery of metal from ores – oxidation of minerals – testing for biodegradability.

UNIT – IV
Biological nitrogen fixation - symbiotic and non-symbiotic microorganisms, root nodule formation, nitrogen fixers, hydrogenase, Nitrogenase, *Nif* gene regulation. Biochemistry of nitrogen fixation, Rhizosphere- R: S ratio, Interaction of microbes with plants. Bioconversion of agricultural wastes. Genetically Modified organisms and crops.

UNIT – V
Biofertilizer - Application of biofertilizers and biomanures – A combination of biofertilizer and manure applications with reference to soil, seed and leaf sprays. Laboratory and field application; Cost-benefit analysis of biofertilizer and biomanure production. Biopesticides and its application.

SUGGESTED READINGS
TEXT BOOKS
1. Subba Rao, N.S. (1999). *Biofertilizers in Agriculture and Agroforestry*. Oxford and IBH, New Delhi.
2. Rangaswami, G., and Bhagyaraj, D.J., (2001). *Agricultural Microbiology*. (2nd ed.). Prentice Hall, New Delhi.
3. Rao, N.S. (1995). *Soil Microorganisms and plant Growth*. Oxford and IBH Publishing Co., New Delhi.
4. Pelzar, M.J., and Reid, M., (2003). *Microbiology*. (5th ed.). Tata Mc Graw-Hill, New York.
5. Reinheimer, G. (1991). *Aquatic Microbiology*. (4th ed.). John Wiley and Sons, New York.

REFERENCES
1. Deniel, J.C. (1996). *Environmental aspects of microbiology*, British Sun Publication, Chennai.
2. Abbasi, S.A. (1998). *Environmental pollution and its control*. Cogent International publishers, Pondicherry.
3. Sen, K., and Ashbolt, N.J., (2010). *Environmental Microbiology: Current Technology and Water Applications.*
4. Josdand, S.N. (1995). *Environmental Biotechnology*. Himalaya Publishing House, Bombay.

5. Maier, R.M., Pepper, I,L., and Gerba, C.P., (2009). *Environmental Microbiology.* (2nd ed.). Elsevier Publisher.
6. Metcalf, R.L., and Luckmann, W.H., (1994). *Introduction to insect pest management.* (3rd ed). John Willey and Sons, Inc.
7. Atlas, R.M., and Bartha, M., (2000). *Microbial Ecology - Fundamental and Applications*. (3rd ed.). Redwood City CA. Benjamin/Cumming Science Publishing Co., New Delhi.
8. Maier, R.M., Pepper, I.L., and Gerba, C.P., (2000). *Environmental Microbiology*. (1st ed.). Academic Press, New York.
9. Mitchell, R. (1992). *Introduction to Environmental Microbiology*; Prentice Hall. Inc. Englewood Clifs- New Jersey.
10. Motsara, M.R., Bhattacharyya, P., and Srivastava, B., (1995). *Biofertilizer- Technology, Marketing and Usage. Fertilizer Development and Consultant Organization*, New Delhi.

LECTURE PLAN

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Microbiology of water	T1- 364-365
2	1	Water pollution and water borne pathogens	T2- 680-681
3	1	Bacteriological examination of water , indicator organism	W1
4	1	Microbiology of sewage	T1- 368-369
5	1	Chemical & biochemical characteristics of sewage	W2
6	1	Methods of sewage treatment- physical screening	T3- 508
7	1	Methods of sewage treatment – chemical screening	T3- 508
8	1	Methods of sewage treatment – biological screening sludge digestion	T3- 508
9	1	Activated sludge, Aerating filters, Oxidation ponds, Unit Revision	T3- 508-521
	Total No of Hours Planned For Unit 1=9		

Textbooks:

T1- Rangaswami.G.A.D.J.Bhagyaraj,2001. Agricultural Microbiology,2nd edition,Prentice Hall,New Delhi.

T2- Sathyanarayana,Victoria, 2005,Biotechnology, Uppala-Publisher interlinks.

T3- Maoer, R.M.,Pepper,I.L.Gerba, C.P., 2009.Emvironmental Microbiology-Second edition,Elsevier Publisher.

W1: https:/en.wikipedia.org/wiki/water-pollution.

W2: www.who.int/water-Sanitation-health/Resource/ quality/wqmchap/0.pdf

LECTURE PLAN

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-II	
1	1	Microbiology of air	T1- 362-363
2	1	Microbial contaminants of air	T3- 105-107
3	1	Source of contaminants	T3- 105-107
4	1	Microbial indicators of air pollution	T3- 113-115
5	1	Microbial indicators of air pollution	T3- 113-115
6	1	Enumeration of bacteria in air	T3- 98-100,114
7	1	Air samplers, Sampling techniques, Air sanitization	T1-. 222-224; T3-103-106,118-119
8	1	Unit Revision	
	Total No of Hours Planned For Unit II=8		

Textbooks:

T1- Rangaswami.G.A.D.J.Bhagyaraj,2001. Agricultural Microbiology,2nd edition,Prentice Hall,New Delhi.

T3- Maoer, R.M.,Pepper,I.L.Gerba, C.P., 2009.Emvironmental Microbiology-Second edition,Elsevier Publisher.

LECTURE PLAN

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-III	
1	1	Introduction to bioremediation	T4: 115-126, W3
2	1	Contaminated soil	T2: 727-729, W4
3	1	Aquifers	T3:. 74, W5:
4	1	Marine pollutants	W6
5	1	Air pollutants	T2, 669,W7:
6	1	Stimulation of oil spills degradation	W8
7	1	Bioremediation of air pollutants	T2: 673-678,
8	1	Biobleaching – recovery of metal from ore	W9:
9	1	Oxidation of minerals, Testing for biodegradability, Unit Revision	W10; T2: 718-724
	Total No of Hours Planned For Unit III=9		

Textbooks:

T2- Sathyanarayana,Victoria, 2005,Biotechnology, Uppala-Publisher interlinks.

T3- Maoer, R.M.,Pepper,I.L.Gerba, C.P., 2009.Emvironmental Microbiology-Second edition,Elsevier Publisher.

T4: Josdand,S.N.,1995.Environmental Biotechnology.Himalaya publishing house,Bombay. 115-126,

W6: http:/en.wikipedia.org/wiki/Aquifer
W7: http:/en.wikipedia.org/wiki/marine pollution
W8: http:/en.wikipedia.org/wiki/air pollution.
W9: http:/en.wikipedia.org/wiki/Microbial degradation.
W10: http:/en.wikipedia.org/Bioleaching

LECTURE PLAN

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-IV	
1	1	Biological nitrogen fixation	R1: 102&108
2	1	Symbiotic microorganism	R1: 115-116
3	1	Non-symbiotic microorganism	R1: : 115-116,
4	1	Root nodule formation	R1: 108, T5: 189-196
5	1	Nitrogen fixers and hydrogenase	T5: 196-199
6	1	Nitrogenase, Nif genes regulation	R1: 108; 113-115
7	1	Biochemistry of nitrogen fixation	R1: 108-112
8	1	Rhizosphere – R:S ratio, Interaction of microbes with plants,	R1: 99-102
9	1	Bioconversion of agricultural wastes, Unit Revision	R1: 116-119
	Total No of Hours Planned For Unit IV=9		

Textbooks:

T5: Subha Rao,N.S (1999) Biofertilisers in Agriculture and Agroforestry, Oxford and IBTT, New Delhi.

References:

R1: Atlas,R.M and M.Bartha,2000.Microbial Ecology,Fundamental and application : 3rd edition. Redwood city CA. Benjamin/Cunning science.Publishing Co. New Delhi.

LECTURE PLAN

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-V	
1	1	Biofertilizers – Introduction	T7: 166-172
2	1	Production of biofertilizer	T7: 166-172
3	1	Application of biofertilizer	T7: 381
4	1	Biomanures	T7: 252-270
5	1	Combination of biofertilizers	T7: 381
6	1	Manure application with reference to soil, seed, leaf sprays	T7: 266
7	1	Laboratory and field application	T7: 270-310 W12:
8	1	Cost benefit analysis of biofertilizer	T7: 166-172
9	1	Biomanure production, Unit Revision	T7: 266
		Total No of Hours Planned for unit V=9	

Textbooks:

T7: Subha Rao. N.S Soil Microbiology.

W12: www.manidharmabiotech.com

Unit 1

Aquatic environment - microbiology of water - water pollution and water borne pathogens. Bacteriological examination of water, indicator organism. Microbiology of sewage. Chemical and biochemical characteristic of sewage. methods of sewage treatment - physical screening, chemical, biological (sludge digestion; activated sludge, aerating filters, oxidation pond).

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

Marine ecosystem

Marine ecosystems cover approximately 71% of the Earth's surface and contain approximately 97% of the planet's water. 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.

Freshwater ecosystem

Freshwater ecosystems cover 0.80% 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

This biota have a size range (maximum linear dimension) up to 200 mm, and vary from viruses through bacteria and archea, to micro-algae, fungi and protozoa.

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.

Microbiology of water

Water microbiology is concerned with the microorganisms that live in the water, or those that can be transported from one habitat to another by water. The improvement of pathogen detection methodology is an important issue for the efficient prevention of waterborne outbreaks. Bacterial populations are a natural component of lakes, rivers, streams and other aquatic systems. Over 60 genera of bacteria are present in aquatic systems and numbers can range from forty thousand to over twelve million bacterial cells in an amount of water. The immense numbers of these small organisms can have an enormous impact on processes that occur in aquatic ecosystems such as carbon, nitrogen, and sulphur transformations. They can also have an impact on the quality of water by controlling the amount of oxygen in the water and causing diseases in aquatic organisms as well as in humans. Naturally some microorganisms have learned to live on or in the human body. Many of these microorganisms do not harm, and are even beneficial because they compete with other microorganisms that might cause diseases. A few microorganisms can cause disease in humans. These microorganisms are called pathogens.Some pathogens live out their lives in the soil and water and only cause disease under unusual circumstances. The microorganism that causes tetanus (a bacterium named Clostridium tetani).This microorganism lives normally in the soil. Other pathogens are more closely associated with humans and other warmblooded animals. These pathogens are transmitted from one organism to another by direct contact, or by contamination of food or water However, the presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enter the water. Contamination of drinking water with a type of *Escherichia coli* known as O157:H7 can be fatal. The contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people.

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

Another group of microbes of concern in water microbiology are protozoa. The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals such as beaver and deer. *Giardia* and *Cryptosporidium* form dormant and hardy forms called cysts during their life cycles. The cyst forms are resistant to chlorine, which is the most popular form of drinking water disinfection, and can pass through the filters used in many water treatment plants. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans, and can be life threatening to those people with impaired immune systems. *Cryptosporidium* contamination of the drinking water of Milwaukee, Wisconsin with in 1993 sickened more than 400,000 people and killed 47 people.

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone. Further away from the shore is the limnitic zone. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Saltwater presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as archaebacteria is one of the dominant forms of life in the ocean. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Another microorganism found in saltwater are a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide" depletes the water of nutrients and oxygen, which can cause many fish to die. As well, humans can become ill by eating contaminated fish.

Water can also be an ideal means of transporting microorganisms from one place to another. For example, the water that is carried in the hulls of ships to stabilize the vessels during their ocean voyages is now known to be a means of transporting microorganisms around the globe. One of these organisms, a bacterium called *Vibrio cholerae*, causes life threatening diarrhea in humans.

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries. For example, in pre-Christian times the storage of drinking water in jugs made of metal was practiced. Now, the anti-bacterial effect of some metals is known. Similarly, the boiling of drinking water, as a means of protection of water has long been known.

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

An important aspect of water microbiology, particularly for drinking water, is the testing of the water to ensure that it is safe to drink. Water quality testing can be done in several ways. One popular test measures the turbidity of the water. Turbidity gives an indication of the amount of suspended material in the water. Typically, if material such as soil is present in the water then microorganisms will also be present. The presence of particles even as small as bacteria and viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating, and so if action should be taken to correct the water problem.

In many countries, water microbiology is also the subject of legislation. Regulations specify how often water sources are sampled, how the sampling is done, how the analysis will be performed, what microbes are detected, and the acceptable limits for the target microorganisms in the water sample. Testing for microbes that cause disease (i.e., *Salmonella typhymurium* and *Vibrio cholerae*) can be expensive and, if the bacteria are present in low numbers, they may escape detection. Instead, other more numerous bacteria provide an indication of fecal pollution of the water. *Escherichia coli* have been used as an indicator of fecal pollution for decades. The bacterium is present in the intestinal tract in huge numbers, and is more numerous than the disease-causing bacteria and viruses. The chances of detecting *Escherichia coli* are better than detecting the actual disease causing microorganisms. *Escherichia coli* also had the advantage of not being capable of growing and reproducing in the water (except in the warm and food-laden waters of tropical countries). Thus, the presence of the bacterium in water is indicative of recent fecal pollution. Finally, *Escherichia coli* can be detected easily and inexpensively.

Water pollution

Definition: Water pollution is characterized by certain observable disturbance in normal properties and functions of fresh water. Eg : includes offensive odour, bad taste etc.,

Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds.

Surface water and groundwater have often been studied and managed as separate resources, although they are interrelated. Surface water seeps through the soil and becomes groundwater.

Ground water pollution

Ground water is considered to be safe and useful for drinking, agricultural and industrial purpose. The specific contaminants leading to pollution in water include chemicals and substances such fluoride, arsenic, nitrate etc., the concentration is the key in determining contaminant. High concentrations of naturally occurring substances can have negative impacts on aquatic flora and fauna and the substances are toxic to humans.

Surface water pollution

Surface water includes rivers, lakes and reservoirs, surface water is susceptible for pollution eg : industrial, domestic, agricultural etc.,

Nature of pollutants

Pollutants may be Dissolved, Suspended,Colloidal in state, they are further categorised as

- Organic pollutants
- Synthetic organic pollutants
- Inorganic pollutants

Organic water pollutants include

Detergents

Disinfection by-products found in chemically disinfected drinking water, such as chloroform Food processing waste, which can include oxygen-demanding substances, fats and grease, Insecticides and herbicides, a huge range of organohalides and other chemical compounds, Petroleum hydrocarbons, including fuels (gasoline, diesel fuel, jet fuels, and fuel oil) and lubricants (motor oil), and fuel combustion byproducts, from storm water runoff, Tree and bush debris from logging operations.

Volatile organic compounds (VOCs)

Chlorinated solvents, which are dense non-aqueous phase liquids (DNAPLs), may fall to the bottom of reservoirs, since they don't mix well with water and are denser.Polychlorinated biphenyl (PCBs).Trichloroethylene. Perchlorate

Various chemical compounds found in personal hygiene and cosmetic products

- Drug pollution involving pharmaceutical drugs and their metabolites.
- Inorganic water pollutants include
- Acidity caused by industrial discharges (especially sulfur dioxide from power plants)
- Ammonia from food processing waste

- Chemical waste as industrial by-products
- Fertilizers containing nutrients--nitrates and phosphates—which are found in stormwater runoff from agriculture, as well as commercial and residential use[16]
- Heavy metals from motor vehicles (via urban stormwater runoff)[16][17] and acid mine drainage

Silt (sediment) in runoff from construction sites, logging, slash and burn practices or land clearing sites.

Microbiological pollutant

Microbiological pollution is caused by wide range of microorganisms like bacteria, viruses , protozo, helminths can cause serious diseases that can lead to death.

Radioactive pollutant

Radioactive contamination, also called radiological contamination, is the deposition of, or presence of radioactive substances on surfaces or within solids, liquids or gases, where their presence is unintended or undesirable. E.g uranium, radium, thorium.

Water borne pathogens

Waterborne diseases are caused by pathogenic microorganisms that most commonly are transmitted in contaminated fresh water. Although the vast majority of bacteria are either harmless or beneficial, a few pathogenic bacteria can cause disease. Coli form bacteria, which are not an actual cause of disease, are commonly used as a bacterial indicator of water pollution. Other microorganisms sometimes found in surface waters that have caused human health problems include:

- *Burkholderia pseudomallei*
- *Cryptosporidium parvum*
- *Giardia lamblia*
- *Salmonella*
- *Norovirus* and other viruses
- *Parasitic worms including the Schistosoma type*

High levels of pathogens may result from on-site sanitation systems (septic tanks, pit latrines) or inadequately treated sewage discharges. This can be caused by a sewage plant designed with less than secondary treatment (more typical in less-developed countries). In developed countries, older cities with aging infrastructure may have leaky sewage collection systems (pipes, pumps, valves), which can cause sanitary sewer overflows. Some cities also have combined sewers, which may discharge untreated sewage during rain storms.

BACTERIAL EXAMINATION OF WATER

The bacteriological examination of water is performed routinely by water utilities to ensure a safe supply of water for drinking, industrial and other domestic uses. The examination is intended to identify water sources which have been contaminated with potential disease causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery. In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The coliform group is comprised of Gram-negative, nonspore-forming, aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. Two organisms in this group include E. coli and Enterobacter aerogenes; however, the only true fecal coliform is E. coli, which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect E. coli. The three principal tests used for bacterial examinations are

- Presumptive test
- Confirmative test
- Complete test

Standard water analysis

The Presumptive test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes.

The confirmed test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. In order the confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram-positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. E. coli and E. aerogenes can be distinguished from one another by the size and color of the colonies. E. coli colonies are small and have a green metallic sheen, whereas E. aerogenes forms large pinkish colonies. If only E. coli or if both

E. coli and E. aerogenes appear on the EMB plate, the test is considered positive. If only E. aerogenes appears on the EMB plate, the test is considered negative. The reasons for these interpretations are that, as previously stated, E. coli is an indicator of fecal contamination, since it is not normally found in water or soil, whereas E. aerogenes is widely distributed in nature outside of the intestinal tract.

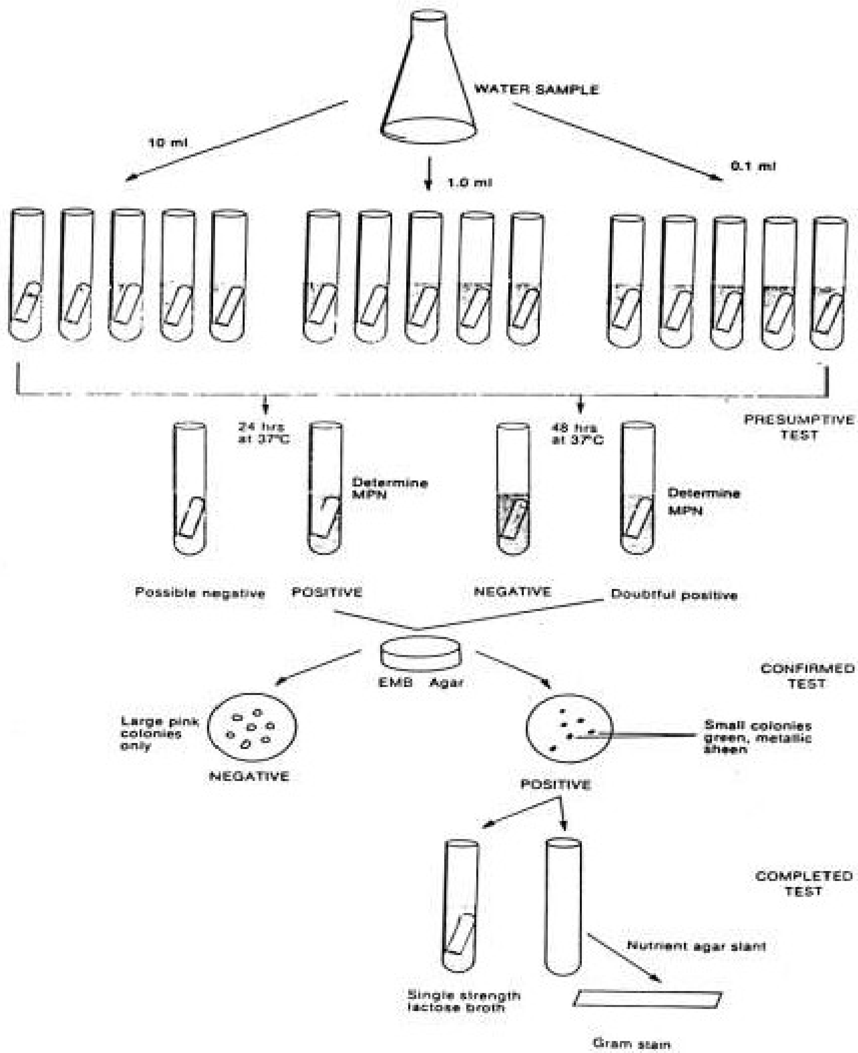
The completed test

The completed test is made using the organisms which grow on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.

The water sample is inoculated in three tubes of lactose broth with 10 ml, three tubes with 1.0 ml and three tubes with 0.1 ml. Incubate all tubes at 37oC for 24 hours. Observe the number of tubes at each dilution that show gas production in 24 hrs. Reincubate for an additional 24 hours at 37°C. Inoculate an EMB plate with material from a tube containing gas. Invert and incubate the plate at 37°C for 24 hours. Observe EMB agar plates. A positive confirmed test is indicated by small colonies with dark centres and a green metallic sheen (E. coli). Inoculate a lactose broth tube and a nutrient agar slant with organisms from the EMB plate. Incubate the broth tube and agar slant at 37°C for 24 hours

MPN DETERMINATION FROM MULTIPLE TUBE TEST

NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF			MPN Index per 100 ml.	95 PERCENT CONFIDENCE LIMITS	
3 of 10 ml. each	3 of 1 ml. each	3 of 0.1 ml. each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800



Indicator organism

Cabelli (1977) noted that the best indicator organism should be the one whose densities correlate best with health hazards associated with one or several given types of pollution sources. The requirements for an indicator as follows:

- The indicator should be consistently and exclusively associated with the source of the pathogens.
- It must be present in sufficient numbers to provide an accurate density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.

- It should approach the resistance to disinfectants and environmental stress, including toxic materials deposited therein, of the most resistant pathogen potentially present at significant levels in the sources.
- It should be quantifiable in recreational waters by reasonably facile and in expensive methods and with considerable accuracy, precision, and specificity.

Indicator organisms indicate that fecal pollution and presences of microbial pathogens. Total and fecal coliforms, and the enterocci - fecal streptocci are important indicator organisms. Coliform bacteria include all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation. There are three groupings of coliform bacteria used as standards: total coliforms (TC), fecal coliforms (FC) and Escherichia coli. Total coliforms are the broadest grouping including Escherichia, Enterobacter, Klebsiella, and Citrobacter. These are found naturally in the soil, as well as in feces. Fecal coliforms are the next widest grouping, which includes many species of bacteria commonly found in the human intestinal tract. Usually between 60% and 90% of total coliforms are fecal coliforms. E. coli are a particular species of bacteria that may or may not be pathogenic but are ubiquitous in the human intestinal tract. Generally more than 90% of the fecal coliform are Escherichia coli.

Microbiology ofsewage

Wastewater, by its nature, is teaming with microbes. Many of these microbes are necessary for the degradation and stabilization of organic matter and thus are beneficial. On the other hand, wastewater may also contain pathogenic or potentially pathogenic microorganisms, which pose a threat to health. Microbes play an extremely important role in sewage treatment. It is largely through biological digestion that sewage is converted from a highly contaminated, infectious liquid into a relatively stable, inert sludge and a harmless effluent needing only chlorination before it may be discharged into a receiving stream, leaching bed, or other disposal area. There are two biological processes involved in sewage treatment.

Aerobic digestion is exemplified by the activated sludge process, in which the wastes from primary settling tanks are thoroughly aerated until active masses of microorganisms settle out as sludge, leaving a clear effluent of relatively low organic content. A portion of the sludge is returned and mixed with the incoming raw sewage, while the remainder is pumped to digester tanks. Anaerobic digestion is a slower process, which is typified by large digestion tanks, septic tanks, and cesspools. The main focus of wastewater treatment plants is to reduce the BOD (biochemical oxygen demand) and COD (chemical oxygen demand) in the effluent discharged to natural waters, meeting state and federal discharge criteria. Wastewater treatment plants are designed to function as "microbiology farms", where bacteria and other microorganisms are fed oxygen and organic wasteewage

Characteristics of Sewage:

Characterization of sewage 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. The wastes are generally characterized as follows:

(i) Physical characteristics: The physical characteristics are colour, odour, turbidity and temperature.

Colour: Fresh sewage is yellowish green or light brown. It is detected by naked eye.

Odour: Fresh sewage is odorless, In 3 to 4 hours, it becomes stale due to exhaust of oxygen in the sewage, mainly due to decomposition of sewage. It is measured by Threshold odour number (TON).

Turbidity: Sewage is generally turbid. If becomes more turbid due to floating matters like floating paper, grease, match sticks, fluid skins etc. Turbidity increases as sewage becomes stronger. Turbidity is measured by turbidity meter.

Temperature: The observation of temperature of sewage is useful in indicating solubility of oxygen, which affects transfer capacity of aeration equipment in aerobic systems and rate of biological activity. Normal temperature of sewage is higher than that of water. Raw temperature of sewage under Indian conditions is between 15-35°C, mostly 20°C.

(ii) Chemical characteristics: Chemical characteristics of sewage help us to find the stage of sewage decomposition, its strength and type of treatment required for safe disposal.

pH: The hydrogen ion concentration expressed as pH, it is valuable parameter in the operation of biological units. Generally pH of raw sewage is in range of 5.5 to 8.0.

Solid matter: Sewage water contains 0.05 to 0.1% of total solids. They are present in four forms

(a) Suspend solids
(b) Dissolved solids
(c) colloidal solids
(d) settle able solids
(e) Organic matter
(f) Inorganic matter.

Chloride content:

Chloride present in sewage from kitchens, urinals, bathrooms & industries. The normal limit is 120 mg/l. The maximum permissible limit is 250mg/l.

Nitrogen content: The presence of nitrogen indicates presence of organic matters. This exists in the form of nitrates, free NH_3 and aluminoides. Nitrate indicates intermediate stage of decomposition. Hence it helps to find the amount of treatment to be done. Natural oxidation prevents nitrates and nitrites.

Dissolved oxygen: Amount of oxygen gas dissolved in sewage water is called as dissolved oxygen. It has to be noted while discharging sewage into stream water. Dissolved content should be more than 4 ppm, else the fishes die and thus affects aquatic cycle. Fresh sewage contains DO in certain amount that gets depleted due to aerobic decomposition. When temperature increases, DO reduces. It is determined by winkler's method

. Biochemical oxygen demand and eutrophication

Organic material in wastewater originates from microorganisms, plants, animals, and synthetic organic compounds. Organic materials enter wastewater in human wastes, paper products, detergents, cosmetics, and foods. They are typically a combination of carbon, hydrogen, oxygen and nitrogen and may contain other elements.

The oxidation of organic materials in the environment can have profound effects on the maintenance of aquatic life and the aesthetic quality of waters. Biochemical oxidation reactions involve the conversion of organic material using oxygen and nutrients into carbon dioxide, water and new cells. The equation that expresses this is:

Organic material + O_2 + nutrients CO_2 + H_2O + new cells + nutrients + energy

It can be seen from this equation that organisms use oxygen to breakdown carbon-based materials for assimilation into new cell mass and energy. A common measure of this oxygen use is biochemical oxygen demand (BOD). BOD is the amount of oxygen used in the metabolism of biodegradable organics. If water with a large amount of BOD is discharged into the environment, it can deplete the natural oxygen resources. Heterotrophic bacteria utilize deposited organics and oxygen at rates that exceed the oxygen-transfer rates across the water surface. This can cause anaerobic conditions, which leads to noxious odors. It can also be detrimental to aquatic life by reducing dissolved oxygen concentrations to levels that cause fish to suffocate. The end result is an overall degradation of water quality.

Wastewater often contains large amounts of the nutrients, particularly nitrogen and phosphorous, which are essential for growth of all organisms and are typically limiting in the environment. Nitrogen is a complex element existing in both organic and inorganic forms. The forms of most interest from a water quality perspective are organic nitrogen, ammonia, nitrite, and nitrate. Phosphorous is found in synthetic detergents and is used for corrosion control in water supplies.

The introduction of large concentrations of these nutrients from untreated or improperly treated wastewater can lead to eutrophication. Eutrophication is the process by which bodies of water become rich in mineral and organic nutrients causing plant life, especially algae, to proliferate, then die and decompose thereby reducing the dissolved oxygen content and often killing off other organisms.

Chemical oxygen demand (COD): The amount of oxygen required to oxidize the organic matter present in sewage (both biologically active and inactive)

Generally COD is greater than BOD. Thus BOD/COD is always less than 1.

.(iii) Biological characteristics: The bacterial characteristics of sewage are due to the presence of micro-organisms, which include bacteria and other living micro-organisms such as algae, fungi, protozoa etc.

Pathogens: it Creates harm to humans, animals, crops.

Non-pathogens: it does not produce harm.

Aerobic bacteria: it survives in the presence of oxygen.

Anaerobic bacteria: Flourish in absence of oxygen.

Facultative bacteria: Survive with or without oxygen.

Most of the microorganisms help in decomposition of sewage.

Sewage Treatment:

Sewage, before being disposed of either in river or streams or on land, has to be treated for making it safe. The degree of treatment required depends on the characteristics of sewage & source disposal. Sewage can be treated in different ways.

The treatment process are often classified as

(i) Preliminary treatment

(ii) Primary treatment

(iii) Secondary treatment

(iv)Tertiary/final treatment (sometimes).

(i) **Preliminary treatment:** It consists of separating the floating materials like dead animals, tree branches, paper, and pieces of wood etc. and also heavy settable inorganic solids. It helps in removing oils and greases etc from sewage. This reduces BOD of waste water by about 15 to 30%. The processes used are

(a) Screening: To remove floating papers, rags, clothes etc.

(b) Grit chamber/Detritus sand: To remove grit and sand

(c) Skimming tanks: To remove oils and greases

(ii) **Primary Treatment**: It consists of removing large suspended organic solids. This is usually done by sedimentation in settling basins. The liquid effluent from primary treatment often contains a large amount of suspended organic material and has high BOD (about 60% of original). The organic solids, which are separated out in sedimentation tanks are stabilized by anaerobic decomposition in digestion tank or incinerated. The residue is used for landfills or soil conditioners.

Secondary Treatment:

Secondary treatment is biological process of very fine suspended matter, colloids and dissolved solids in sewage that comes from primary sedimentation tank.

The treatment stabilizes and makes the sewage completely harmless.

The unit process of secondary treatment are biological oxidation and synthesis through sewage filter or activated sludge process, converting sewage into heavier and bulkier and then allowing it to settle in secondary sedimentation tank.

The separated sewage sludge is decomposed anaerobically in sludge tank and the digested sludge is disposed off separately in sludge drying beds.

Difference between primary treatment and secondary treatment of sewage

(i) Primary treatment:

It is evident that the sewage as it arrives at the treatment plant would initially undergo primary treatment for the removal of heavy suspended matter such as solids, kitchen refuse, cloth, wastepaper etc.

Inorganic matter like sand, grit and other floating matter.

The primary treatment involves subjecting sewage subsequently to unit processes such as screening. Screening is the removal of grit and other floating matter and the remaining solids are removed through sedimentation process in primary sedimentation tank or clarifier. The primary treatment removes the physical impurities present in sewage along with solid matter. Sometimes preliminary treatment is also termed along with the primary treatment.

(ii) Secondary Treatment:

Secondary treatment involves further treatment of the effluent coming from the primary sedimentation tank. This is generally accomplished through biological decomposition of organic matter, under aerobic or anaerobic conditions. Bacteria get decomposed to fine organic matter, to produce clearer effluent.

(i) Aerobic biological unit: Under aerobic conditions eg: filters, aeration tanks, oxidation ponds, aerated lagoons

(ii) Anaerobic biological unit: Under anaerobic conditions eg: anaerobic lagoons, septic tanks etc.

Benefits of sewage treatment:

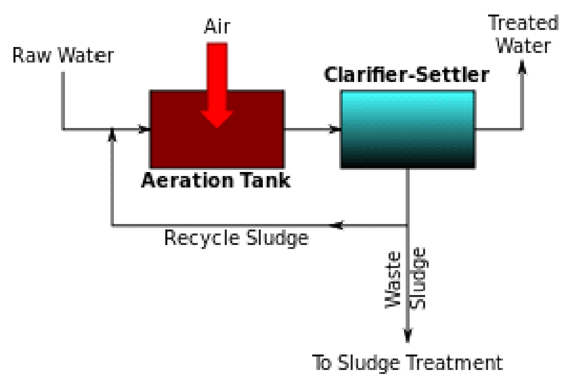
Save money by recycling a portion of waste water for use around garden.

Limit the impact of house waste on environment by becoming more self sufficient.

Protects precious source of ground water and saves rainwater in dams by recycling.

Reduce impact on municipal sewage system by installing domestic treatment system, particularly grey water treatment system.

Methods of sewage treatment



Activated sludge

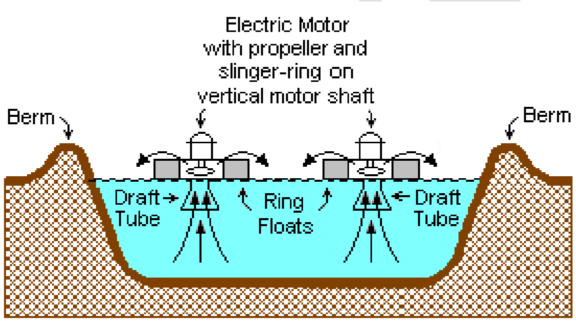
Activated sludge plants encompass a variety of mechanisms and processes that use dissolved oxygen to promote the growth of biological floc that substantially removes organic material. Biological floc, as mentioned above, is an ecosystem of living biota that subsists on nutrients from the inflowing primary settling tank (or clarifier) effluent. These mostly carbonaceous dissolved solids undergo aeration to be broken down and biologically oxidized or converted to carbon dioxide. Likewise, nitrogenous dissolved solids (amino acids, ammonia, etc.) are also oxidized by the floc to nitrites, nitrates, and, in some processes, to nitrogen gas through denitrification. While denitrification is encouraged in some treatment processes, in many suspended aeration plants denitrification will impair the settling of the floc and lead to poor quality effluent. In either case, the settled floc is both recycled to the inflowing primary effluent to regrow, or is partially 'wasted' (or diverted) to solids dewatering, or digesting, and then dewatering. This many times takes the form of the floating brown foam, Nocardia. While this so called 'sewage fungus' (it isn't really a fungus) is the best known, there are many different fungi and protists that can overpopulate the floc and cause process upsets. Additionally, certain incoming chemical species, such as a heavy pesticide, a heavy metal (eg.: plating company effluent) load, or extreme pH, can kill the biota of an activated sludge reactor ecosystem. Such problems are tested for, and if caught in time, can be neutralized.

Aerobic granular sludge

Activated sludge systems can be transformed into aerobic granular sludge systems (aerobic granulation) which enhance the benefits of activated sludge, like increased biomass retention due to high sludge settlability.

Surface-aerated basins

Many small municipal sewage systems in the United States (1 million gal./day or less) use aerated lagoons.Most biological oxidation processes for treating industrial wastewaters have in common the use of oxygen (or air) and microbial action. Surface-aerated basins achieve 80 to 90 percent removal of BOD with retention times of 1 to 10 days. The basins may range in depth from 1.5 to 5.0 metres and use motor-driven aerators floating on the surface of the wastewater. In an aerated basin system, the aerators provide two functions: they transfer air into the basins required by the biological oxidation reactions, and they provide the mixing required for dispersing the air and for contacting the reactants (that is, oxygen, wastewater and microbes). Typically, the floating surface aerators are rated to deliver the amount of air equivalent to 1.8 to 2.7 kg O2/kW·h. However, they do not provide as good mixing as is normally achieved in activated sludge systems and therefore aerated basins do not achieve the same performance level as activated sludge units.Biological oxidation processes are sensitive to temperature and, between 0 °C and 40 °C, the rate of biological reactions increase with temperature. Most surface aerated vessels operate at between 4 °C and 32 °C.



A TYPICAL SURFACE – AERATED BASIN

Note: The ring floats are tethered to posts on the berms.

Filter beds

Trickling filter beds are used where the settled sewage liquor is spread onto the surface of a bed made up of coke (carbonized coal), limestone chips or specially fabricated plastic media. Such media must have large surface areas to support the biofilms that form. The liquor is typically distributed through perforated spray arms. The distributed liquor trickles through the bed and is collected in drains at the base. These drains also provide a source of air which percolates up through the bed, keeping it aerobic. Biological films of bacteria, protozoa and fungi form on the media's surfaces and eat or otherwise reduce the organic content. This biofilm is often grazed by insect larvae, snails, and worms which help maintain an optimal thickness. Overloading of beds increases the thickness of the film leading to clogging of the filter media and ponding on the surface. Recent advances in media and process micro-biology design overcome many issues with trickling filter designs.

Biological aerated filters

Biological Aerated (or Anoxic) Filter (BAF) or Biofilters combine filtration with biological carbon reduction, nitrification or denitrification. BAF usually includes a reactor filled with a filter media. The media is either in suspension or supported by a gravel layer at the foot of the filter. The dual purpose of this media is to support highly active biomass that is attached to it and to filter suspended solids. Carbon reduction and ammonia conversion occurs in aerobic mode and sometime achieved in a single reactor while nitrate conversion occurs in anoxic mode. BAF is operated either in upflow or downflow configuration depending on design specified by manufacturer.

Rotating biological contactors

Rotating biological contactors (RBCs) are mechanical secondary treatment systems, which are robust and capable of withstanding surges in organic load. RBCs were first installed in Germany in 1960 and have since been developed and refined into a reliable operating unit. The rotating disks support the growth of bacteria and micro-organisms present in the sewage, which break down and stabilize organic pollutants. To be successful, micro-organisms need both oxygen to live and food to grow. Oxygen is obtained from the atmosphere as the disks rotate. As the micro-organisms grow, they build up on the media until they are sloughed off due to shear forces provided by the rotating discs in the sewage. Effluent from the RBC is then passed through final clarifiers where the micro-organisms in suspension settle as a sludge. The sludge is withdrawn from the clarifier for further treatment.

A functionally similar biological filtering system has become popular as part of home aquarium filtration and purification. The aquarium water is drawn up out of the tank and then cascaded over a freely spinning corrugated fiber-mesh wheel before passing through a media filter and back into the aquarium. The spinning mesh wheel develops a biofilm coating of microorganisms that feed on the suspended wastes in the aquarium water and are also exposed to the atmosphere as the wheel rotates. This is especially good at removing waste urea and ammonia urinated into the aquarium water by the fish and other animals.

Membrane bioreactors

Membrane bioreactors (MBR) combine activated sludge treatment with a membrane liquid-solid separation process. The membrane component uses low pressure microfiltration or ultrafiltration membranes and eliminates the need for clarification and tertiary filtration. The membranes are typically immersed in the aeration tank; however, some applications utilize a separate membrane tank. One of the key benefits of an MBR system is that it effectively overcomes the limitations associated with poor settling of sludge in conventional activated sludge (CAS) processes. The technology permits bioreactor operation with considerably higher mixed liquor suspended solids (MLSS) concentration than CAS systems, which are limited by sludge settling. The process is typically operated at MLSS in the range of 8,000–12,000 mg/L, while CAS are operated in the range of 2,000–3,000 mg/L. The elevated biomass concentration in the MBR process allows for very effective removal of both soluble and particulate biodegradable materials at higher loading rates. Thus increased sludge retention times, usually exceeding 15 days, ensure complete nitrification even in extremely cold weather.

The cost of building and operating an MBR is often higher than conventional methods of sewage treatment. Membrane filters can be blinded with grease or abraded by suspended grit and lack a clarifier's flexibility to pass peak flows. The technology has become increasingly popular for reliably pretreated waste streams and has gained wider acceptance where infiltration and inflow have been controlled, however, and the life-cycle costs have been steadily decreasing. The small footprint of MBR systems, and the high quality effluent produced, make them particularly useful for water reuse applications.

Secondary sedimentation

The final step in the secondary treatment stage is to settle out the biological floc or filter material through a secondary clarifier and to produce sewage water containing low levels of organic material and suspended matter.

Tertiary treatment

The purpose of tertiary treatment is to provide a final treatment stage to further improve the effluent quality before it is discharged to the receiving environment (sea, river, lake, wet lands,ground, etc.). More than one tertiary treatment process may be used at any treatment plant. If disinfection is practiced, it is always the final process. It is also called "effluent polishing."

Filtration

Sand filtration removes much of the residual suspended matter. Filtration over activated carbon, also called carbon adsorption, removes residual toxins.

Lagooning

Lagooning provides settlement and further biological improvement through storage in large man-made ponds or lagoons. These lagoons are highly aerobic and colonization by native macrophytes, especially reeds, is often encouraged. Small filter feeding invertebrates such as Daphnia and species of Rotifera greatly assist in treatment by removing fine particulates.

Nutrient removal

Wastewater may contain high levels of the nutrients nitrogen and phosphorus. Excessive release to the environment can lead to a buildup of nutrients, called eutrophication, which can in turn encourage the overgrowth of weeds, algae, and cyanobacteria (blue-green algae). This may cause an algal bloom, a rapid growth in the population of algae. The algae numbers are unsustainable and eventually most of them die. The decomposition of the algae by bacteria uses up so much of the oxygen in the water that most or all of the animals die, which creates more organic matter for the bacteria to decompose. In addition to causing deoxygenation, some algal species produce toxins that contaminate drinking water supplies. Different treatment processes are required to remove nitrogen and phosphorus.

Nitrogen removal

The removal of nitrogen is effected through the biological oxidation of nitrogen from ammonia to nitrate (nitrification), followed by denitrification, the reduction of nitrate to nitrogen gas. Nitrogen gas is released to the atmosphere and thus removed from the water.

Nitrification itself is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia (NH_3) to nitrite (NO_2^-) is most often facilitated by Nitrosomonas spp. ("nitroso" referring to the formation of a nitroso functional group). Nitrite oxidation to nitrate (NO_3^-), though traditionally believed to be facilitated by Nitrobacter spp. (nitro referring the formation of a nitro functional group), is now known to be facilitated in the environment almost exclusively by Nitrospira spp.

Denitrification requires anoxic conditions to encourage the appropriate biological communities to form. It is facilitated by a wide diversity of bacteria. Sand filters, lagooning and reed beds can all be used to reduce nitrogen, but the activated sludge process (if designed well) can do the job the most easily. Since denitrification is the reduction of nitrate to dinitrogen gas, an electron donor is needed. This can be, depending on the wastewater, organic matter (from faeces), sulfide, or an added donor like methanol. The sludge in the anoxic tanks (denitrification tanks) must be mixed well (mixture of recirculated mixed liquor, return activated sludge [RAS], and raw influent) e.g. by using submersible mixers in order to achieve the desired denitrification.

Phosphorus removal

Phosphorus can be removed biologically in a process called enhanced biological phosphorus removal. In this process, specific bacteria, called polyphosphate-accumulating organisms (PAOs), are selectively enriched and accumulate large quantities of phosphorus within their cells (up to 20 percent of their mass). When the biomass enriched in these bacteria is separated from the treated water, these biosolids have a high fertilizer value. Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g. ferric chloride), aluminum (e.g. alum), or lime. This may lead to excessive sludge production as hydroxides precipitates and the added chemicals can be expensive. Chemical phosphorus removal requires significantly smaller equipment footprint than biological removal, is easier to operate and is often more reliable than biological phosphorus removal.

Disinfection

The purpose of disinfection in the treatment of waste water is to substantially reduce the number of microorganisms in the water to be discharged back into the environment for the later use of drinking, bathing, irrigation, etc. The effectiveness of disinfection depends on the quality of the water being treated (e.g., cloudiness, pH, etc.), the type of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Cloudy water will be treated less successfully, since solid matter can shield organisms, especially from ultraviolet light or if contact times are low. Generally, short contact times, low doses and high flows all militate against effective disinfection. Common methods of disinfection include ozone, chlorine, ultraviolet light, or sodium hypochlorite. Chloramine, which is used for drinking water, is not used in the treatment of waste water because of its persistence. After multiple steps of disinfection, the treated water is ready to be released back into the water cycle by means of the nearest body of water or agriculture. Afterwards, the water can be transferred to reserves for everyday human uses.

Chlorination remains the most common form of waste water disinfection in North America due to its low cost and long-term history of effectiveness. One disadvantage is that chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual chlorine or chloramines may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated, adding to the complexity and cost of treatment.

Ultraviolet (UV) light can be used instead of chlorine, iodine, or other chemicals. Because no chemicals are used, the treated water has no adverse effect on organisms that later consume it, as may be the case with other methods. UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens, making them incapable of reproduction. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement and the need for a highly treated effluent to ensure that the target microorganisms are not shielded from the UV radiation (i.e., any solids present in the treated effluent may protect microorganisms from the UV light). In the United Kingdom, UV light is becoming the most common means of disinfection because of the concerns about the impacts of chlorine in chlorinating residual organics in the wastewater and in chlorinating organics in the receiving water. Some sewage treatment systems in Canada and the US also use UV light for their effluent water disinfection.

Ozone (O3) is generated by passing oxygen (O2) through a high voltage potential resulting in a third oxygen atom becoming attached and forming O3. Ozone is very unstable and reactive and oxidizes most organic material it comes in contact with, thereby destroying many pathogenic microorganisms. Ozone is considered to be safer than chlorine because, unlike chlorine which has to be stored on site (highly poisonous in the event of an accidental release), ozone is generated onsite as needed. Ozonation also produces fewer disinfection by-products than chlorination. A disadvantage of ozone disinfection is the high cost of the ozone generation equipment and the requirements for special operators.

Benefits of sewage treatment:

- Save money by recycling a portion of waste water for use around garden.
- Limit the impact of house waste on environment by becoming more self sufficient.
- Protects precious source of ground water and saves rainwater in dams by recycling.
- Reduce impact on municipal sewage system by installing domestic treatment system, particularly grey water treatment system.

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write an account of water born pathogen ?
2. Give the steps of typical sewage treatment plant
3. What is biochemical oxygen demand (BOD)?
4. What is a trickling filter?
5. What is MPN?

PART-C (8 MARKS)

1. Explain the water pollution
2. Write the detailed notes about the bacterial examination of water?
3. Explain the physical, chemical and biological methods of Sewage treatment
4. Give a detailed note on characters of sewage
5. Giv a notes on sludge digestion; activated sludge, aerating filters, oxidation pond

S.No	Unit I	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	_______ filters provide a high surface area to grow a biomass	Carbon	Diatom	Aerated	Micro	Aerated
2	_______ is a semiconductor photo catalyst.	Sodium hypochloride solution	Resorcinol	Triethylene glycol	Titanium dioxide	Titanium dioxide
3	________ virus causes whooping cough.	*Varicella*	*Influenza*	*Bordetella pertussis*	*Rubella*	*Bordetella pertussis*
4	_________ is a semiconductor photo catalyst.	Sodium hypochloride solution	Resorcinol	Triethylene glycol	Titanium dioxide	Titanium dioxide
5	__________________are relatively more abundant than the vegetative cells in the air	Spores	Infectious dust	Aerosols	Droplets	Spores
6	_____________is an occupational disease	Brucellosis	Pulmanory disease	Pneumonitis	Meningitis	Brucellosis
7	___________can be a source of infectious diseases	Droplets	Aerosols	Dust	Flocs	Aerosols
8	Air borne infections are transmitted mainly by	Aerobes from person to person	Inhaling spores or hyphal fragments from soil or dead vegetation	Drinking contaminated water	Objects such as handkerchiefs that are contaminated with respiratory secretations	Aerobes from person to person
9	Air doesn't have a__flora	Indigenous	Auctothonous	Normal	None of the above	Normal
10	Airborne particles are a major cause of ________ allergies in humans.	Gastrointestinal	Urinary tract	Respiratory	Eye	Respiratory
11	Average salinity of seawater	15 ppt	25 ppt	35 ppt	45 ppt	35 ppt
12	Bacteriological examination of water usually employs	Total count	Multiple tube method	Membrane filters count	Plate count	Multiple tube method
13	*Chlorella pyrenoidosa is usually found in*	Activated	Sludge compost	Trickling filter	Oxidation	Activated

		sludge process			pond	sludge process
14	Copper is used in water treatement as a	Disinfectant	Indicator	Coagulant	Flocculants	Disinfectant
15	Droplet nuclei are significant in the transmission of diseases of the	Digestive system	Nervous system	Reproductive system	Respiratory system	Respiratory system
16	Ecological region at the lowest level of a body of water such as an ocean or a lake	Limnetic zone	Littoral zone	Profoundal zone	Benthic zone	Benthic zone
17	Effective air sanitizing is done by	Gamma radiation	UV radiation	Beta radiation	Gamma radiation	UV radiation
18	Elemental sulphur to sulphuric acid oxidised by ---------	Algae	Bacteria	Fungi	Viruses	Bacteria
19	Farmer's lung caused by exposure to spores of thermophilic ________	Fungi	Bacteria	Actinomycetes	Viruses	Actinomycetes
20	Fomites are	Insect vectors	Inanimate objects	Animate objects	Biological vectors	Inanimate objects
21	Formation of-------- is crucial step in anaerobic digestion	Hydrogen	Carbondioxide	Water	Acetate	Acetate
22	HEPA filters are typically rated as ________ effective in removing dust.	90	99.92%	99.97%	90.99%	99.97%
23	In a lake the combined littoral and limnetic zone is known as	Profundal zone	Euphotic zone	Metalimnon	Epilimnon	Epilimnon
24	In Lemon sampler air is drawn at the rate of______________per minute and dispersed through the broth	20-25litre	25-30litre	30-35litre	35-40litre	20-25litre
25	Laminar airflow developed by __________.	Whittaker	Whitifield	Tyndall	Koch	Whitifield
26	Lilton large volume air sampler is an example for ___________.	Sieve sampler	Impingement	Electrostatic precipitation	Centrifugal action	Electrostatic precipitation
27	Main photosynthetic body of the lake	Littoral zone	Limnetic zone	Profoundal zone	Paleic zone	Limnetic zone
28	Marine ecosystems cover approximately _____ of the Earth's surface	7%	71%	17%	77%	71%
29	Microbes in air can be enumerated by	Settle plate method	Pour plate mehod	Spread plate method	Streak plate method	Settle plate method

30	Microorganisms found attached to the rock surface are reffered to as	Epipeplon	Episammon	Epixylon	Epilithon	Epipeplon
31	Of the different atmospheric layers ________is characterized by a heavy load of microorganisms	Troposphere	Stratosphere	Lithosphere	Atmosphere	Troposphere
32	Profoundal zone is	Open surface of water body	Sub-surface zone	Deepest zone	Side zone	Deepest zone
33	Relative humidity for survival of the microorganism is between -----------	25-45 percent	40-80 percent	40-60 percent	50-70 percent	40-80 percent
34	Schmutzdecke is a hypogeal biological layer formed on surface of slow sand filter by	Fungi	Bacteria	Protozoa	Algae	Bacteria
35	Slit sampler can collect upto ______% of the water droplet particles sprayed into air	85%	100%	95%	75%	85%
36	Sludge conditioning is accomplished by which of the following	Thickening	Elutriation	Chemical conditioning	Diluting with water	Thickening
37	Spores of ___________ travel over a thousand kilometers.	*Clostridium perfringens*	*Puccinia graminis*	*Sarcina lutea*	*Micrococcus luteus*	*Clostridium perfringens*
38	The amount of carbondioxide present in the atmosphere is near to	0.02%	0.03%	0.04%	0.05%	0.04%
39	The dominant genera of common saprophytic fungi in indoor air is	Aspergillus	Fusarium	Penicillium	Mucor	Penicillium
40	The filtering medium of the tank becomes coated with a microbial flora, the __________ film	Biofilm	Zoogloeal film	.Neustonic	Algal bloom	Zoogloeal film
41	The most commonly efficient substrate used as a carbon source indenitrification during sewage treatement is	Methanol	Oxygen	Glucose	Sucrose	Methanol
42	The optimum rate of relative humidity for the survival of the most microorganisms is	40-80%	60-80%	50-80%	30-80%	40-80%
43	Viruses survive in the atmosphere at low temperature from	8 to 32°C	7 to 24°C	6 to 18°C	2 to 6°C	6 to 18°C
44	Which of the following can be seen in marine environment?	Halophiles	Barophiles	Psychrophiles	Hydrophiles	Halophiles

45	Which of the following is not an aerobic process?	Activated sludge process	Sludge digestion	Trickling filter	Oxidation pond	Trickling filter
46	Which of the following is not common in marine environment?	Luminous bacteria	Psychrophilic bacteria	Thermophilic bacteria	Barophilic bacteria	Luminous bacteria
47	Zone near shore area where sunlight penetrates the sediment and allows aquatic plants to grow	Littoral zone	Limnetic zone	Paleic zone	Profoundal zone	Littoral zone
48	Zoogloeal film formed in the trickling filter consists of	Bacteria	Algae	Protozoa	Algal bloom	Bacteria
49	Which of the following is the type of endosymbiosis	Commensalisms	cooperation	mutualism	predation	Commensalism
50	hich of the following microorganism grows well at temperatures above boiling point of water	Halobacterium	Methanococcus jannaschii	Pyrococcus furiosis	Bacillus	Pyrococcus furiosis
51	Which of the following method is used for removal of suspended materials from waste water	Filration	Purification	sedimentation	settlement	Sedimentation
52	Most of the indicator oranisms for detection of disease occurrence level in drinking water belongs to which of the following microroganisms group	Actinobacteria	Bacilli	Coliform	Firmicutes	Coliform
53	Which among the following microbes is not a prime concern for deterioration of water quality in drinking water	Legionella	Shigella	Vibrio parahaemolyticus	Vibrio vulnificus	Shigella
54	Which of the following test is used as preusmptive test for enumeration of coliform in water samples?	Most probable number	Heterocoliformcount	Aerobic colony count	colony forming unit	Most probable number
55	Which of the following promotes the biological transformation of dissolved organic matter to microbial biomass and carbondioxide	Primary	Secondary	Tertiary	Quaternary	Secondary
56	Inorganic nutrients are removed by biological means refer as which of the following treatment process	Primary	Secondary	Tertiary	Quaternary	Tertiary
57	In industrial processing plants, which of the following is the principle factor of treating waste water	Removal of microbes	Removal of organics	Removal of solids	Removal of liquids	Removal of organics
58	Along with inorganic and organic nutrients which of the following compounds are removed through tertiary treatment process	Heavy and trace metals	Lignocellulosic	Suspended matters	Floating materials	Heavy and trace metals

59	Zone near shore area where sunlight penetrates the sediment and allows aquatic plants to grow	Littoral zone	Limnetic zone	Paleic zone	Profoundal zone	Littoral zone
60	Microbes in air can be enumerated by	Settle plate method	Pour plate mehod	Spread plate method	Streak plate method	Settle plate method

Unit 1

Aquatic environment - microbiology of water - water pollution and water borne pathogens. Bacteriological examination of water, indicator organism. Microbiology of sewage. Chemical and biochemical characteristic of sewage. methods of sewage treatment - physical screening, chemical, biological (sludge digestion; activated sludge, aerating filters, oxidation pond).

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

Marine ecosystem

Marine ecosystems cover approximately 71% of the Earth's surface and contain approximately 97% of the planet's water. 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.

Freshwater ecosystem

Freshwater ecosystems cover 0.80% 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

This biota have a size range (maximum linear dimension) up to 200 mm, and vary from viruses through bacteria and archea, to micro-algae, fungi and protozoa.

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.

Microbiology of water

Water microbiology is concerned with the microorganisms that live in the water, or those that can be transported from one habitat to another by water. The improvement of pathogen detection methodology is an important issue for the efficient prevention of waterborne outbreaks. Bacterial populations are a natural component of lakes, rivers, streams and other aquatic systems. Over 60 genera of bacteria are present in aquatic systems and numbers can range from forty thousand to over twelve million bacterial cells in an amount of water. The immense numbers of these small organisms can have an enormous impact on processes that occur in aquatic ecosystems such as carbon, nitrogen, and sulphur transformations. They can also have an impact on the quality of water by controlling the amount of oxygen in the water and causing diseases in aquatic organisms as well as in humans. Naturally some microorganisms have learned to live on or in the human body. Many of these microorganisms do not harm, and are even beneficial because they compete with other microorganisms that might cause diseases. A few microorganisms can cause disease in humans. These microorganisms are called pathogens.Some pathogens live out their lives in the soil and water and only cause disease under unusual circumstances. The microorganism that causes tetanus (a bacterium named Clostridium tetani).This microorganism lives normally in the soil. Other pathogens are more closely associated with humans and other warmblooded animals. These pathogens are transmitted from one organism to another by direct contact, or by contamination of food or water However, the presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enter the water. Contamination of drinking water with a type of *Escherichia coli* known as O157:H7 can be fatal. The contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people.

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

Another group of microbes of concern in water microbiology are protozoa. The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals such as beaver and deer. *Giardia* and *Cryptosporidium* form dormant and hardy forms called cysts during their life cycles. The cyst forms are resistant to chlorine, which is the most popular form of drinking water disinfection, and can pass through the filters used in many water treatment plants. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans, and can be life threatening to those people with impaired immune systems. *Cryptosporidium* contamination of the drinking water of Milwaukee, Wisconsin with in 1993 sickened more than 400,000 people and killed 47 people.

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone. Further away from the shore is the limnitic zone. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Saltwater presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as archaebacteria is one of the dominant forms of life in the ocean. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Another microorganism found in saltwater are a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide" depletes the water of nutrients and oxygen, which can cause many fish to die. As well, humans can become ill by eating contaminated fish.

Water can also be an ideal means of transporting microorganisms from one place to another. For example, the water that is carried in the hulls of ships to stabilize the vessels during their ocean voyages is now known to be a means of transporting microorganisms around the globe. One of these organisms, a bacterium called *Vibrio cholerae*, causes life threatening diarrhea in humans.

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries. For example, in pre-Christian times the storage of drinking water in jugs made of metal was practiced. Now, the anti-bacterial effect of some metals is known. Similarly, the boiling of drinking water, as a means of protection of water has long been known.

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

An important aspect of water microbiology, particularly for drinking water, is the testing of the water to ensure that it is safe to drink. Water quality testing can be done in several ways. One popular test measures the turbidity of the water. Turbidity gives an indication of the amount of suspended material in the water. Typically, if material such as soil is present in the water then microorganisms will also be present. The presence of particles even as small as bacteria and viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating, and so if action should be taken to correct the water problem.

In many countries, water microbiology is also the subject of legislation. Regulations specify how often water sources are sampled, how the sampling is done, how the analysis will be performed, what microbes are detected, and the acceptable limits for the target microorganisms in the water sample. Testing for microbes that cause disease (i.e., *Salmonella typhymurium* and *Vibrio cholerae*) can be expensive and, if the bacteria are present in low numbers, they may escape detection. Instead, other more numerous bacteria provide an indication of fecal pollution of the water. *Escherichia coli* have been used as an indicator of fecal pollution for decades. The bacterium is present in the intestinal tract in huge numbers, and is more numerous than the disease-causing bacteria and viruses. The chances of detecting *Escherichia coli* are better than detecting the actual disease causing microorganisms. *Escherichia coli* also had the advantage of not being capable of growing and reproducing in the water (except in the warm and food-laden waters of tropical countries). Thus, the presence of the bacterium in water is indicative of recent fecal pollution. Finally, *Escherichia coli* can be detected easily and inexpensively.

Water pollution

Definition: Water pollution is characterized by certain observable disturbance in normal properties and functions of fresh water. Eg : includes offensive odour, bad taste etc.,

Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds.

Surface water and groundwater have often been studied and managed as separate resources, although they are interrelated. Surface water seeps through the soil and becomes groundwater.

Ground water pollution

Ground water is considered to be safe and useful for drinking, agricultural and industrial purpose. The specific contaminants leading to pollution in water include chemicals and substances such fluoride, arsenic, nitrate etc., the concentration is the key in determining contaminant. High concentrations of naturally occurring substances can have negative impacts on aquatic flora and fauna and the substances are toxic to humans.

Surface water pollution

Surface water includes rivers, lakes and reservoirs, surface water is susceptible for pollution eg : industrial, domestic, agricultural etc.,

Nature of pollutants

Pollutants may be Dissolved, Suspended,Colloidal in state, they are further categorised as

- Organic pollutants
- Synthetic organic pollutants
- Inorganic pollutants

Organic water pollutants include

Detergents

Disinfection by-products found in chemically disinfected drinking water, such as chloroform Food processing waste, which can include oxygen-demanding substances, fats and grease, Insecticides and herbicides, a huge range of organohalides and other chemical compounds, Petroleum hydrocarbons, including fuels (gasoline, diesel fuel, jet fuels, and fuel oil) and lubricants (motor oil), and fuel combustion byproducts, from storm water runoff, Tree and bush debris from logging operations.

Volatile organic compounds (VOCs)

Chlorinated solvents, which are dense non-aqueous phase liquids (DNAPLs), may fall to the bottom of reservoirs, since they don't mix well with water and are denser.Polychlorinated biphenyl (PCBs).Trichloroethylene. Perchlorate

Various chemical compounds found in personal hygiene and cosmetic products

- Drug pollution involving pharmaceutical drugs and their metabolites.
- Inorganic water pollutants include
- Acidity caused by industrial discharges (especially sulfur dioxide from power plants)
- Ammonia from food processing waste

- Chemical waste as industrial by-products
- Fertilizers containing nutrients--nitrates and phosphates—which are found in stormwater runoff from agriculture, as well as commercial and residential use[16]
- Heavy metals from motor vehicles (via urban stormwater runoff)[16][17] and acid mine drainage

Silt (sediment) in runoff from construction sites, logging, slash and burn practices or land clearing sites.

Microbiological pollutant

Microbiological pollution is caused by wide range of microorganisms like bacteria, viruses , protozo, helminths can cause serious diseases that can lead to death.

Radioactive pollutant

Radioactive contamination, also called radiological contamination, is the deposition of, or presence of radioactive substances on surfaces or within solids, liquids or gases, where their presence is unintended or undesirable. E.g uranium, radium, thorium.

Water borne pathogens

Waterborne diseases are caused by pathogenic microorganisms that most commonly are transmitted in contaminated fresh water. Although the vast majority of bacteria are either harmless or beneficial, a few pathogenic bacteria can cause disease. Coli form bacteria, which are not an actual cause of disease, are commonly used as a bacterial indicator of water pollution. Other microorganisms sometimes found in surface waters that have caused human health problems include:

- *Burkholderia pseudomallei*
- *Cryptosporidium parvum*
- *Giardia lamblia*
- *Salmonella*
- *Norovirus* and other viruses
- *Parasitic worms including the Schistosoma type*

High levels of pathogens may result from on-site sanitation systems (septic tanks, pit latrines) or inadequately treated sewage discharges. This can be caused by a sewage plant designed with less than secondary treatment (more typical in less-developed countries). In developed countries, older cities with aging infrastructure may have leaky sewage collection systems (pipes, pumps, valves), which can cause sanitary sewer overflows. Some cities also have combined sewers, which may discharge untreated sewage during rain storms.

BACTERIAL EXAMINATION OF WATER

The bacteriological examination of water is performed routinely by water utilities to ensure a safe supply of water for drinking, industrial and other domestic uses. The examination is intended to identify water sources which have been contaminated with potential disease causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery. In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The coliform group is comprised of Gram-negative, nonspore-forming, aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. Two organisms in this group include E. coli and Enterobacter aerogenes; however, the only true fecal coliform is E. coli, which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect E. coli. The three principal tests used for bacterial examinations are

- Presumptive test
- Confirmative test
- Complete test

Standard water analysis

The Presumptive test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes.

The confirmed test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. In order the confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram-positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. E. coli and E. aerogenes can be distinguished from one another by the size and color of the colonies. E. coli colonies are small and have a green metallic sheen, whereas E. aerogenes forms large pinkish colonies. If only E. coli or if both

E. coli and E. aerogenes appear on the EMB plate, the test is considered positive. If only E. aerogenes appears on the EMB plate, the test is considered negative. The reasons for these interpretations are that, as previously stated, E. coli is an indicator of fecal contamination, since it is not normally found in water or soil, whereas E. aerogenes is widely distributed in nature outside of the intestinal tract.

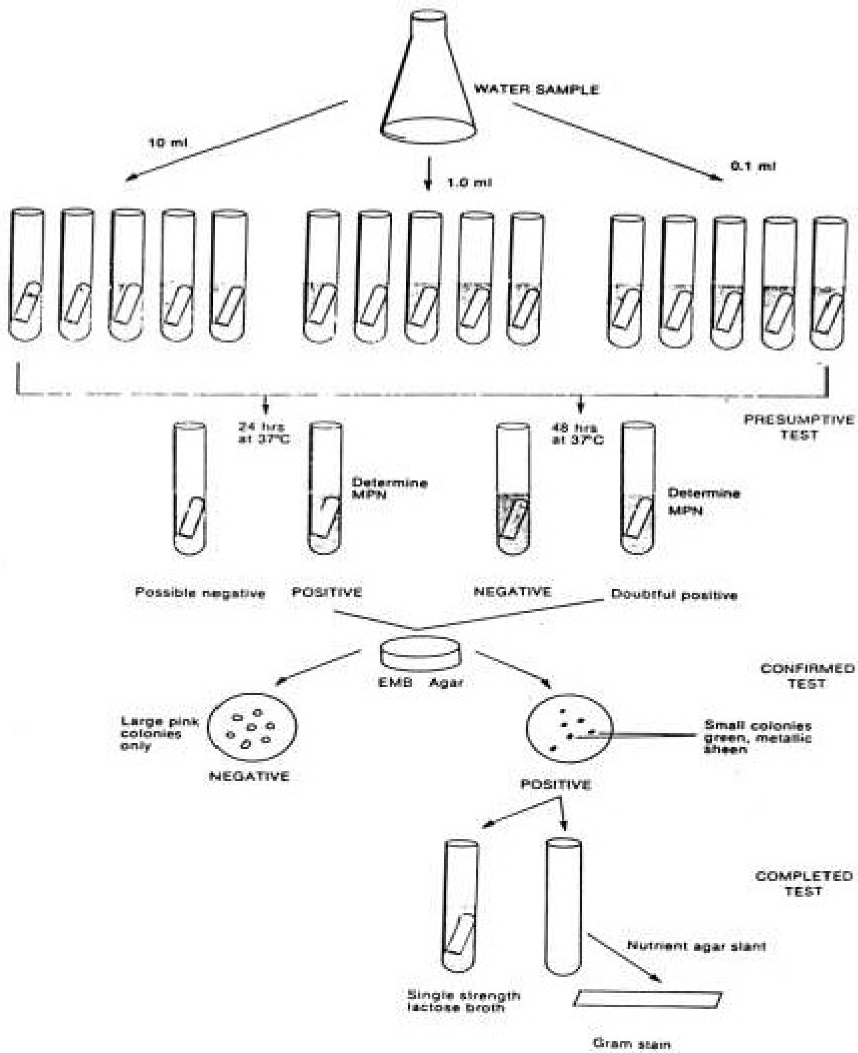
The completed test

The completed test is made using the organisms which grow on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.

The water sample is inoculated in three tubes of lactose broth with 10 ml, three tubes with 1.0 ml and three tubes with 0.1 ml. Incubate all tubes at 37oC for 24 hours. Observe the number of tubes at each dilution that show gas production in 24 hrs. Reincubate for an additional 24 hours at 37°C. Inoculate an EMB plate with material from a tube containing gas. Invert and incubate the plate at 37°C for 24 hours. Observe EMB agar plates. A positive confirmed test is indicated by small colonies with dark centres and a green metallic sheen (E. coli). Inoculate a lactose broth tube and a nutrient agar slant with organisms from the EMB plate. Incubate the broth tube and agar slant at 37°C for 24 hours

MPN DETERMINATION FROM MULTIPLE TUBE TEST

NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF			MPN Index per 100 ml.	95 PERCENT CONFIDENCE LIMITS	
3 of 10 ml. each	3 of 1 ml. each	3 of 0.1 ml. each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800



Indicator organism

Cabelli (1977) noted that the best indicator organism should be the one whose densities correlate best with health hazards associated with one or several given types of pollution sources. The requirements for an indicator as follows:

- The indicator should be consistently and exclusively associated with the source of the pathogens.
- It must be present in sufficient numbers to provide an accurate density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.

- It should approach the resistance to disinfectants and environmental stress, including toxic materials deposited therein, of the most resistant pathogen potentially present at significant levels in the sources.
- It should be quantifiable in recreational waters by reasonably facile and in expensive methods and with considerable accuracy, precision, and specificity.

Indicator organisms indicate that fecal pollution and presences of microbial pathogens. Total and fecal coliforms, and the enterocci - fecal streptocci are important indicator organisms. Coliform bacteria include all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation. There are three groupings of coliform bacteria used as standards: total coliforms (TC), fecal coliforms (FC) and Escherichia coli. Total coliforms are the broadest grouping including Escherichia, Enterobacter, Klebsiella, and Citrobacter. These are found naturally in the soil, as well as in feces. Fecal coliforms are the next widest grouping, which includes many species of bacteria commonly found in the human intestinal tract. Usually between 60% and 90% of total coliforms are fecal coliforms. E. coli are a particular species of bacteria that may or may not be pathogenic but are ubiquitous in the human intestinal tract. Generally more than 90% of the fecal coliform are Escherichia coli.

Microbiology ofsewage

Wastewater, by its nature, is teaming with microbes. Many of these microbes are necessary for the degradation and stabilization of organic matter and thus are beneficial. On the other hand, wastewater may also contain pathogenic or potentially pathogenic microorganisms, which pose a threat to health. Microbes play an extremely important role in sewage treatment. It is largely through biological digestion that sewage is converted from a highly contaminated, infectious liquid into a relatively stable, inert sludge and a harmless effluent needing only chlorination before it may be discharged into a receiving stream, leaching bed, or other disposal area. There are two biological processes involved in sewage treatment.

Aerobic digestion is exemplified by the activated sludge process, in which the wastes from primary settling tanks are thoroughly aerated until active masses of microorganisms settle out as sludge, leaving a clear effluent of relatively low organic content. A portion of the sludge is returned and mixed with the incoming raw sewage, while the remainder is pumped to digester tanks. Anaerobic digestion is a slower process, which is typified by large digestion tanks, septic tanks, and cesspools. The main focus of wastewater treatment plants is to reduce the BOD (biochemical oxygen demand) and COD (chemical oxygen demand) in the effluent discharged to natural waters, meeting state and federal discharge criteria. Wastewater treatment plants are designed to function as "microbiology farms", where bacteria and other microorganisms are fed oxygen and organic wasteewage

Characteristics of Sewage:

Characterization of sewage 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. The wastes are generally characterized as follows:

(i) Physical characteristics: The physical characteristics are colour, odour, turbidity and temperature.

Colour: Fresh sewage is yellowish green or light brown. It is detected by naked eye.

Odour: Fresh sewage is odorless, In 3 to 4 hours, it becomes stale due to exhaust of oxygen in the sewage, mainly due to decomposition of sewage. It is measured by Threshold odour number (TON).

Turbidity: Sewage is generally turbid. If becomes more turbid due to floating matters like floating paper, grease, match sticks, fluid skins etc. Turbidity increases as sewage becomes stronger. Turbidity is measured by turbidity meter.

Temperature: The observation of temperature of sewage is useful in indicating solubility of oxygen, which affects transfer capacity of aeration equipment in aerobic systems and rate of biological activity. Normal temperature of sewage is higher than that of water. Raw temperature of sewage under Indian conditions is between 15-35°C, mostly 20°C.

(ii) Chemical characteristics: Chemical characteristics of sewage help us to find the stage of sewage decomposition, its strength and type of treatment required for safe disposal.

pH: The hydrogen ion concentration expressed as pH, it is valuable parameter in the operation of biological units. Generally pH of raw sewage is in range of 5.5 to 8.0.

Solid matter: Sewage water contains 0.05 to 0.1% of total solids. They are present in four forms

(a) Suspend solids
(b) Dissolved solids
(c) colloidal solids
(d) settle able solids
(e) Organic matter
(f) Inorganic matter.

Chloride content:

Chloride present in sewage from kitchens, urinals, bathrooms & industries. The normal limit is 120 mg/l. The maximum permissible limit is 250mg/l.

Nitrogen content: The presence of nitrogen indicates presence of organic matters. This exists in the form of nitrates, free NH_3 and aluminoides. Nitrate indicates intermediate stage of decomposition. Hence it helps to find the amount of treatment to be done. Natural oxidation prevents nitrates and nitrites.

Dissolved oxygen: Amount of oxygen gas dissolved in sewage water is called as dissolved oxygen. It has to be noted while discharging sewage into stream water. Dissolved content should be more than 4 ppm, else the fishes die and thus affects aquatic cycle. Fresh sewage contains DO in certain amount that gets depleted due to aerobic decomposition. When temperature increases, DO reduces. It is determined by winkler's method

. Biochemical oxygen demand and eutrophication

Organic material in wastewater originates from microorganisms, plants, animals, and synthetic organic compounds. Organic materials enter wastewater in human wastes, paper products, detergents, cosmetics, and foods. They are typically a combination of carbon, hydrogen, oxygen and nitrogen and may contain other elements.

The oxidation of organic materials in the environment can have profound effects on the maintenance of aquatic life and the aesthetic quality of waters. Biochemical oxidation reactions involve the conversion of organic material using oxygen and nutrients into carbon dioxide, water and new cells. The equation that expresses this is:

Organic material + O_2 + nutrients CO_2 + H_2O + new cells + nutrients + energy

It can be seen from this equation that organisms use oxygen to breakdown carbon-based materials for assimilation into new cell mass and energy. A common measure of this oxygen use is biochemical oxygen demand (BOD). BOD is the amount of oxygen used in the metabolism of biodegradable organics. If water with a large amount of BOD is discharged into the environment, it can deplete the natural oxygen resources. Heterotrophic bacteria utilize deposited organics and oxygen at rates that exceed the oxygen-transfer rates across the water surface. This can cause anaerobic conditions, which leads to noxious odors. It can also be detrimental to aquatic life by reducing dissolved oxygen concentrations to levels that cause fish to suffocate. The end result is an overall degradation of water quality.

Wastewater often contains large amounts of the nutrients, particularly nitrogen and phosphorous, which are essential for growth of all organisms and are typically limiting in the environment. Nitrogen is a complex element existing in both organic and inorganic forms. The forms of most interest from a water quality perspective are organic nitrogen, ammonia, nitrite, and nitrate. Phosphorous is found in synthetic detergents and is used for corrosion control in water supplies.

The introduction of large concentrations of these nutrients from untreated or improperly treated wastewater can lead to eutrophication. Eutrophication is the process by which bodies of water become rich in mineral and organic nutrients causing plant life, especially algae, to proliferate, then die and decompose thereby reducing the dissolved oxygen content and often killing off other organisms.

Chemical oxygen demand (COD): The amount of oxygen required to oxidize the organic matter present in sewage (both biologically active and inactive)

Generally COD is greater than BOD. Thus BOD/COD is always less than 1.

.(iii) Biological characteristics: The bacterial characteristics of sewage are due to the presence of micro-organisms, which include bacteria and other living micro-organisms such as algae, fungi, protozoa etc.

Pathogens: it Creates harm to humans, animals, crops.

Non-pathogens: it does not produce harm.

Aerobic bacteria: it survives in the presence of oxygen.

Anaerobic bacteria: Flourish in absence of oxygen.

Facultative bacteria: Survive with or without oxygen.

Most of the microorganisms help in decomposition of sewage.

Sewage Treatment:

Sewage, before being disposed of either in river or streams or on land, has to be treated for making it safe. The degree of treatment required depends on the characteristics of sewage & source disposal. Sewage can be treated in different ways.

The treatment process are often classified as

(i) Preliminary treatment

(ii) Primary treatment

(iii) Secondary treatment

(iv)Tertiary/final treatment (sometimes).

(i) **Preliminary treatment:** It consists of separating the floating materials like dead animals, tree branches, paper, and pieces of wood etc. and also heavy settable inorganic solids. It helps in removing oils and greases etc from sewage. This reduces BOD of waste water by about 15 to 30%. The processes used are

(a) Screening: To remove floating papers, rags, clothes etc.

(b) Grit chamber/Detritus sand: To remove grit and sand

(c) Skimming tanks: To remove oils and greases

(ii) **Primary Treatment**: It consists of removing large suspended organic solids. This is usually done by sedimentation in settling basins. The liquid effluent from primary treatment often contains a large amount of suspended organic material and has high BOD (about 60% of original). The organic solids, which are separated out in sedimentation tanks are stabilized by anaerobic decomposition in digestion tank or incinerated. The residue is used for landfills or soil conditioners.

Secondary Treatment:

Secondary treatment is biological process of very fine suspended matter, colloids and dissolved solids in sewage that comes from primary sedimentation tank.

The treatment stabilizes and makes the sewage completely harmless.

The unit process of secondary treatment are biological oxidation and synthesis through sewage filter or activated sludge process, converting sewage into heavier and bulkier and then allowing it to settle in secondary sedimentation tank.

The separated sewage sludge is decomposed anaerobically in sludge tank and the digested sludge is disposed off separately in sludge drying beds.

Difference between primary treatment and secondary treatment of sewage

(i) Primary treatment:

It is evident that the sewage as it arrives at the treatment plant would initially undergo primary treatment for the removal of heavy suspended matter such as solids, kitchen refuse, cloth, wastepaper etc.

Inorganic matter like sand, grit and other floating matter.

The primary treatment involves subjecting sewage subsequently to unit processes such as screening. Screening is the removal of grit and other floating matter and the remaining solids are removed through sedimentation process in primary sedimentation tank or clarifier. The primary treatment removes the physical impurities present in sewage along with solid matter. Sometimes preliminary treatment is also termed along with the primary treatment.

(ii) Secondary Treatment:

Secondary treatment involves further treatment of the effluent coming from the primary sedimentation tank. This is generally accomplished through biological decomposition of organic matter, under aerobic or anaerobic conditions. Bacteria get decomposed to fine organic matter, to produce clearer effluent.

(i) Aerobic biological unit: Under aerobic conditions eg: filters, aeration tanks, oxidation ponds, aerated lagoons

(ii) Anaerobic biological unit: Under anaerobic conditions eg: anaerobic lagoons, septic tanks etc.

Benefits of sewage treatment:

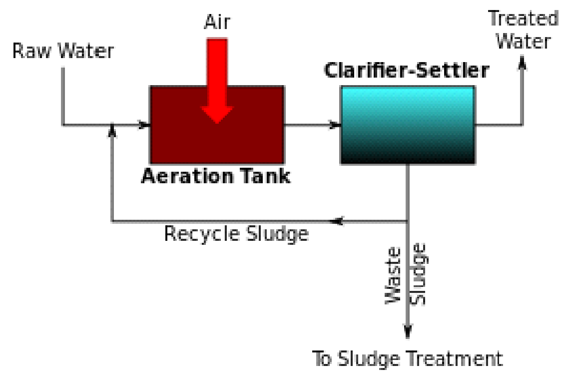
Save money by recycling a portion of waste water for use around garden.

Limit the impact of house waste on environment by becoming more self sufficient.

Protects precious source of ground water and saves rainwater in dams by recycling.

Reduce impact on municipal sewage system by installing domestic treatment system, particularly grey water treatment system.

Methods of sewage treatment



Activated sludge

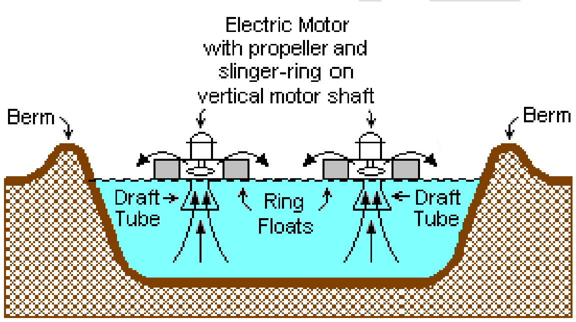
Activated sludge plants encompass a variety of mechanisms and processes that use dissolved oxygen to promote the growth of biological floc that substantially removes organic material. Biological floc, as mentioned above, is an ecosystem of living biota that subsists on nutrients from the inflowing primary settling tank (or clarifier) effluent. These mostly carbonaceous dissolved solids undergo aeration to be broken down and biologically oxidized or converted to carbon dioxide. Likewise, nitrogenous dissolved solids (amino acids, ammonia, etc.) are also oxidized by the floc to nitrites, nitrates, and, in some processes, to nitrogen gas through denitrification. While denitrification is encouraged in some treatment processes, in many suspended aeration plants denitrification will impair the settling of the floc and lead to poor quality effluent. In either case, the settled floc is both recycled to the inflowing primary effluent to regrow, or is partially 'wasted' (or diverted) to solids dewatering, or digesting, and then dewatering. This many times takes the form of the floating brown foam, Nocardia. While this so called 'sewage fungus' (it isn't really a fungus) is the best known, there are many different fungi and protists that can overpopulate the floc and cause process upsets. Additionally, certain incoming chemical species, such as a heavy pesticide, a heavy metal (eg.: plating company effluent) load, or extreme pH, can kill the biota of an activated sludge reactor ecosystem. Such problems are tested for, and if caught in time, can be neutralized.

Aerobic granular sludge

Activated sludge systems can be transformed into aerobic granular sludge systems (aerobic granulation) which enhance the benefits of activated sludge, like increased biomass retention due to high sludge settlability.

Surface-aerated basins

Many small municipal sewage systems in the United States (1 million gal./day or less) use aerated lagoons.Most biological oxidation processes for treating industrial wastewaters have in common the use of oxygen (or air) and microbial action. Surface-aerated basins achieve 80 to 90 percent removal of BOD with retention times of 1 to 10 days. The basins may range in depth from 1.5 to 5.0 metres and use motor-driven aerators floating on the surface of the wastewater. In an aerated basin system, the aerators provide two functions: they transfer air into the basins required by the biological oxidation reactions, and they provide the mixing required for dispersing the air and for contacting the reactants (that is, oxygen, wastewater and microbes). Typically, the floating surface aerators are rated to deliver the amount of air equivalent to 1.8 to 2.7 kg O2/kW·h. However, they do not provide as good mixing as is normally achieved in activated sludge systems and therefore aerated basins do not achieve the same performance level as activated sludge units.Biological oxidation processes are sensitive to temperature and, between 0 °C and 40 °C, the rate of biological reactions increase with temperature. Most surface aerated vessels operate at between 4 °C and 32 °C.



A TYPICAL SURFACE – AERATED BASIN

Note: The ring floats are tethered to posts on the berms.

Filter beds

Trickling filter beds are used where the settled sewage liquor is spread onto the surface of a bed made up of coke (carbonized coal), limestone chips or specially fabricated plastic media. Such media must have large surface areas to support the biofilms that form. The liquor is typically distributed through perforated spray arms. The distributed liquor trickles through the bed and is collected in drains at the base. These drains also provide a source of air which percolates up through the bed, keeping it aerobic. Biological films of bacteria, protozoa and fungi form on the media's surfaces and eat or otherwise reduce the organic content. This biofilm is often grazed by insect larvae, snails, and worms which help maintain an optimal thickness. Overloading of beds increases the thickness of the film leading to clogging of the filter media and ponding on the surface. Recent advances in media and process micro-biology design overcome many issues with trickling filter designs.

Biological aerated filters

Biological Aerated (or Anoxic) Filter (BAF) or Biofilters combine filtration with biological carbon reduction, nitrification or denitrification. BAF usually includes a reactor filled with a filter media. The media is either in suspension or supported by a gravel layer at the foot of the filter. The dual purpose of this media is to support highly active biomass that is attached to it and to filter suspended solids. Carbon reduction and ammonia conversion occurs in aerobic mode and sometime achieved in a single reactor while nitrate conversion occurs in anoxic mode. BAF is operated either in upflow or downflow configuration depending on design specified by manufacturer.

Rotating biological contactors

Rotating biological contactors (RBCs) are mechanical secondary treatment systems, which are robust and capable of withstanding surges in organic load. RBCs were first installed in Germany in 1960 and have since been developed and refined into a reliable operating unit. The rotating disks support the growth of bacteria and micro-organisms present in the sewage, which break down and stabilize organic pollutants. To be successful, micro-organisms need both oxygen to live and food to grow. Oxygen is obtained from the atmosphere as the disks rotate. As the micro-organisms grow, they build up on the media until they are sloughed off due to shear forces provided by the rotating discs in the sewage. Effluent from the RBC is then passed through final clarifiers where the micro-organisms in suspension settle as a sludge. The sludge is withdrawn from the clarifier for further treatment.

A functionally similar biological filtering system has become popular as part of home aquarium filtration and purification. The aquarium water is drawn up out of the tank and then cascaded over a freely spinning corrugated fiber-mesh wheel before passing through a media filter and back into the aquarium. The spinning mesh wheel develops a biofilm coating of microorganisms that feed on the suspended wastes in the aquarium water and are also exposed to the atmosphere as the wheel rotates. This is especially good at removing waste urea and ammonia urinated into the aquarium water by the fish and other animals.

Membrane bioreactors

Membrane bioreactors (MBR) combine activated sludge treatment with a membrane liquid-solid separation process. The membrane component uses low pressure microfiltration or ultrafiltration membranes and eliminates the need for clarification and tertiary filtration. The membranes are typically immersed in the aeration tank; however, some applications utilize a separate membrane tank. One of the key benefits of an MBR system is that it effectively overcomes the limitations associated with poor settling of sludge in conventional activated sludge (CAS) processes. The technology permits bioreactor operation with considerably higher mixed liquor suspended solids (MLSS) concentration than CAS systems, which are limited by sludge settling. The process is typically operated at MLSS in the range of 8,000–12,000 mg/L, while CAS are operated in the range of 2,000–3,000 mg/L. The elevated biomass concentration in the MBR process allows for very effective removal of both soluble and particulate biodegradable materials at higher loading rates. Thus increased sludge retention times, usually exceeding 15 days, ensure complete nitrification even in extremely cold weather.

The cost of building and operating an MBR is often higher than conventional methods of sewage treatment. Membrane filters can be blinded with grease or abraded by suspended grit and lack a clarifier's flexibility to pass peak flows. The technology has become increasingly popular for reliably pretreated waste streams and has gained wider acceptance where infiltration and inflow have been controlled, however, and the life-cycle costs have been steadily decreasing. The small footprint of MBR systems, and the high quality effluent produced, make them particularly useful for water reuse applications.

Secondary sedimentation

The final step in the secondary treatment stage is to settle out the biological floc or filter material through a secondary clarifier and to produce sewage water containing low levels of organic material and suspended matter.

Tertiary treatment

The purpose of tertiary treatment is to provide a final treatment stage to further improve the effluent quality before it is discharged to the receiving environment (sea, river, lake, wet lands,ground, etc.). More than one tertiary treatment process may be used at any treatment plant. If disinfection is practiced, it is always the final process. It is also called "effluent polishing."

Filtration

Sand filtration removes much of the residual suspended matter. Filtration over activated carbon, also called carbon adsorption, removes residual toxins.

Lagooning

Lagooning provides settlement and further biological improvement through storage in large man-made ponds or lagoons. These lagoons are highly aerobic and colonization by native macrophytes, especially reeds, is often encouraged. Small filter feeding invertebrates such as Daphnia and species of Rotifera greatly assist in treatment by removing fine particulates.

Nutrient removal

Wastewater may contain high levels of the nutrients nitrogen and phosphorus. Excessive release to the environment can lead to a buildup of nutrients, called eutrophication, which can in turn encourage the overgrowth of weeds, algae, and cyanobacteria (blue-green algae). This may cause an algal bloom, a rapid growth in the population of algae. The algae numbers are unsustainable and eventually most of them die. The decomposition of the algae by bacteria uses up so much of the oxygen in the water that most or all of the animals die, which creates more organic matter for the bacteria to decompose. In addition to causing deoxygenation, some algal species produce toxins that contaminate drinking water supplies. Different treatment processes are required to remove nitrogen and phosphorus.

Nitrogen removal

The removal of nitrogen is effected through the biological oxidation of nitrogen from ammonia to nitrate (nitrification), followed by denitrification, the reduction of nitrate to nitrogen gas. Nitrogen gas is released to the atmosphere and thus removed from the water.

Nitrification itself is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia ($NH3$) to nitrite ($NO2^-$) is most often facilitated by Nitrosomonas spp. ("nitroso" referring to the formation of a nitroso functional group). Nitrite oxidation to nitrate ($NO3^-$), though traditionally believed to be facilitated by Nitrobacter spp. (nitro referring the formation of a nitro functional group), is now known to be facilitated in the environment almost exclusively by Nitrospira spp.

Denitrification requires anoxic conditions to encourage the appropriate biological communities to form. It is facilitated by a wide diversity of bacteria. Sand filters, lagooning and reed beds can all be used to reduce nitrogen, but the activated sludge process (if designed well) can do the job the most easily. Since denitrification is the reduction of nitrate to dinitrogen gas, an electron donor is needed. This can be, depending on the wastewater, organic matter (from faeces), sulfide, or an added donor like methanol. The sludge in the anoxic tanks (denitrification tanks) must be mixed well (mixture of recirculated mixed liquor, return activated sludge [RAS], and raw influent) e.g. by using submersible mixers in order to achieve the desired denitrification.

Phosphorus removal

Phosphorus can be removed biologically in a process called enhanced biological phosphorus removal. In this process, specific bacteria, called polyphosphate-accumulating organisms (PAOs), are selectively enriched and accumulate large quantities of phosphorus within their cells (up to 20 percent of their mass). When the biomass enriched in these bacteria is separated from the treated water, these biosolids have a high fertilizer value. Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g. ferric chloride), aluminum (e.g. alum), or lime. This may lead to excessive sludge production as hydroxides precipitates and the added chemicals can be expensive. Chemical phosphorus removal requires significantly smaller equipment footprint than biological removal, is easier to operate and is often more reliable than biological phosphorus removal.

Disinfection

The purpose of disinfection in the treatment of waste water is to substantially reduce the number of microorganisms in the water to be discharged back into the environment for the later use of drinking, bathing, irrigation, etc. The effectiveness of disinfection depends on the quality of the water being treated (e.g., cloudiness, pH, etc.), the type of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Cloudy water will be treated less successfully, since solid matter can shield organisms, especially from ultraviolet light or if contact times are low. Generally, short contact times, low doses and high flows all militate against effective disinfection. Common methods of disinfection include ozone, chlorine, ultraviolet light, or sodium hypochlorite. Chloramine, which is used for drinking water, is not used in the treatment of waste water because of its persistence. After multiple steps of disinfection, the treated water is ready to be released back into the water cycle by means of the nearest body of water or agriculture. Afterwards, the water can be transferred to reserves for everyday human uses.

Chlorination remains the most common form of waste water disinfection in North America due to its low cost and long-term history of effectiveness. One disadvantage is that chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual chlorine or chloramines may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated, adding to the complexity and cost of treatment.

Ultraviolet (UV) light can be used instead of chlorine, iodine, or other chemicals. Because no chemicals are used, the treated water has no adverse effect on organisms that later consume it, as may be the case with other methods. UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens, making them incapable of reproduction. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement and the need for a highly treated effluent to ensure that the target microorganisms are not shielded from the UV radiation (i.e., any solids present in the treated effluent may protect microorganisms from the UV light). In the United Kingdom, UV light is becoming the most common means of disinfection because of the concerns about the impacts of chlorine in chlorinating residual organics in the wastewater and in chlorinating organics in the receiving water. Some sewage treatment systems in Canada and the US also use UV light for their effluent water disinfection.

Ozone (O3) is generated by passing oxygen (O2) through a high voltage potential resulting in a third oxygen atom becoming attached and forming O3. Ozone is very unstable and reactive and oxidizes most organic material it comes in contact with, thereby destroying many pathogenic microorganisms. Ozone is considered to be safer than chlorine because, unlike chlorine which has to be stored on site (highly poisonous in the event of an accidental release), ozone is generated onsite as needed. Ozonation also produces fewer disinfection by-products than chlorination. A disadvantage of ozone disinfection is the high cost of the ozone generation equipment and the requirements for special operators.

Benefits of sewage treatment:

- Save money by recycling a portion of waste water for use around garden.
- Limit the impact of house waste on environment by becoming more self sufficient.
- Protects precious source of ground water and saves rainwater in dams by recycling.
- Reduce impact on municipal sewage system by installing domestic treatment system, particularly grey water treatment system.

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write an account of water born pathogen ?
2. Give the steps of typical sewage treatment plant
3. What is biochemical oxygen demand (BOD)?
4. What is a trickling filter?
5. What is MPN?

PART-C (8 MARKS)

1. Explain the water pollution
2. Write the detailed notes about the bacterial examination of water?
3. Explain the physical, chemical and biological methods of Sewage treatment
4. Give a detailed note on characters of sewage
5. Giv a notes on sludge digestion; activated sludge, aerating filters, oxidation pond

S.No	Unit II	Opt 1	Opt 2	Opt 3	Opt 4	Answers
1	________ is a seasonal disease that can be deadly when becomes pandemic	Tuberculosis	Leprosy	Food poisoning	Influenza	Influenza
2	_________ air sampler is used in electrostatic precipitation of removing particles from air	Impinger	Orange	Sutton small volume	Litton large volume	Litton large volume
3	_________ is a severe respiratory disease caused by bacteria	Aspergillosis	Scarlet fever	Tuberculosis	Tetanus	Tuberculosis
4	__________ is an opportunistic fungal disease of human caused by inhalation of spores	Sporidiasis	Penicilliosis	Aspergillosis	Candidiasis	Aspergillosis
5	______________ is transmitted to humans by inhalation of faecal dust from pigeon droppings	Candidiasis	Histoplasmosis	Sporidiasis	Bacteraemia	Histoplasmosis
6	According to ambient air quality standards in India, amount of suspended particulate matters in a residential area is	200 µgm-3	400 µgm-3	600 µgm-3	800 µgm-3	200 µgm-3
7	Air doesn't have ___________ flora	Indigenous	Endogenous	Exogenous	Subgenous	Indigenous
8	Common source of air microflora is	Human	Soil	Industries	Vehicles	Both I & II
9	Cryptococcosis is caused by	*C.neoformans*	*C.licheniformis*	*C.pseudopodis*	*C.parvum*	*C.neoformans*
10	Efficiency of HEPA filter	75%	90%	95%	99.97%	99.97%
11	HEPA is an	Water filter	Soil filter	Air filter	Smoke arrester	Air filter
12	HEPA stands for	High Efficiency Particulate Air	High Energy Particulate Air	High Emission Particulate Air	High Efficient Polar Aerosol	High Efficiency Particulate Air
13	Human impact on the environment is termed as	Metagenic	Mutagenic	Carcinogenic	Anthropogenic	Anthropogenic
14	In India, amount of Carbon monoxide level permissible under ambient air quality standards for an industrial area is	5000 µgm-3	500 µgm-3	50 µgm-3	5 µgm-3	5000 µgm-3
15	Optimum rate of relative humidity for the survival of most microorganisms is between	10-20%	30-60%	40-80 %	80-100%	40-80 %
16	Percentage of oxygen is atmospheric air	40.9	60.9	75.9	20.9	20.9
17	Residue of solid material left after drying up of a droplet is known as	Mucus	Karyon	Oocyst	Droplet nuclei	Droplet nuclei
18	Size of a droplet nuclei	0.1-0.4 µm	1-4 µm	4-10 µm	10-40 µm	1-4 µm

19	Spores of _______________ travel over thousand kilometers	*Blastomyces*	*Fusarium*	*Puccinia graminis*	*Aspergillus*	*Puccinia graminis*
20	The commonest genera of fungi in indoor air are	*Saccharomyces*	*Penicillium*	*Rhizopus*	*Mucor*	*Penicillium*
21	The dominant microflora of outside air are	Fungi	Bacteria	Algae	Virus	Fungi
22	The layer nearest to the earth is called as	Troposphere	Ionosphere	Stratosphere	Thermosphere	Troposphere
23	Transmission of _____________ is mainly by inhaling the dust contaminated by animal products	Cholera	Anthrax	Typhoid	Dysentry	Anthrax
24	Valley fever or desert rheumatism is caused by	*Bordetella pertusis*	*Legionella pneuphila*	*Bacillus anthracis*	*Coccidioides immitis*	*Coccidioides immitis*
25	Vapours of _________ are strongly germicidal	Poly ethylene glycol	Propylene glycol	Glycerol	Glycerin	Propylene glycol
26	Wavelength most effective in air sanitation by UV	254 nm	354 nm	454 nm	545 nm	254 nm
27	Which gas dominates composition of air?	CO_2	Oxygen	Nitrogen	Hydrogen	Nitrogen
28	Which gas has greatest effect on global warming?	Ethane	Methane	CO_2	Hydrogen	Methane
29	Which has greatest effect on ozone layer?	NO_2	SO_2	CFC	CO_2	Chloro fluoro carbon
30	Which has the least presence in air?	Nitrogen	Oxygen	Argon	CO_2	CO_2
31	All are particulate pollutants except	dust	ozone	soot	Smoke	ozone
32	Fine organic and inorganic particles suspended in air is called ___________	Particulate pollutant	gaseous pollutant	aerosol	none of these	aerosol
33	Which of the following is a secondary pollutant	CO_2	CO	O_3	SO_2	O_3
34	Carbonmonoxide is a pollutant because	it reacts with O_2	It inhibits glycolysis	makes nervous system inactive	Reacts with haemoglobin	Reacts with haemoglobin
35	Air pollution is severe in _____________	Cities	Agricultural field	Schools	Desert	Cities
36	The major pollutant from automobile exhaust is _________	NO_2	CO	SO_2	Soot	CO

37	A high biological oxygen demand	Water is pure	absence of microbial action	Low level of microbial pollution	High level of microbial pollution	High level of microbial pollution
38	Algal bloom results in _________	Global warming	Salination	Eutrophication	Biomagnification	Eutrophication
39	The green house gases, otherwise called radioactively active gases includes__________	CO_2	O_2	NO_2	NO	CO_2
40	According to EPA of USA , the following is not one of the six major pollutants?	Ozone	carbonmoxide	nitrogen oxides	carbondioxide	carbondioxide
41	Which of the following is an organic gas?	Hydrocarbons	Aldehydes	Ketones	Ammonia	Ammonia
42	Ozone is formed in the upper atmosphere by a photochemical reaction with ______	Ultraviolet solar radiation	Infra red radiation	Visible light	Gamma rays	Ultra violet solar radiation
43	Identify the term that describes an environment completely free of microorganisms	Antibiotic	Asepsis	Antisepsis	Probiotic	Asepsis
44	Human impact on the environment is termed as	Metagenic	Mutagenic	Carcinogenic	Anthropogenic	Anthropogenic
45	The major pollutant from automobile exhaust is ________	NO_2	CO	SO_2	Soot	CO
46	Size of a droplet nuclei	0.1-0.4 µm	1-4 µm	4-10 µm	10-40 µm	1-4 µm
47	Air doesn't have ___________ flora	Indigenous	Endogenous	Exogenous	Subgenous	Indigenous
48	The major pollutant from automobile exhaust is ________	NO_2	CO	SO_2	Soot	CO
49	The commonest genera of fungi in indoor air are	*Saccharomyces*	*Penicillium*	*Rhizopus*	*Mucor*	*Penicillium*
50	______________ is transmitted to humans by inhalation of faecal dust from pigeon droppings	Candidiasis	Histoplasmosis	Sporidiasis	Bacteraemia	Histoplasmosis
51	Ozone is formed in the upper atmosphere by a photochemical reaction with ______	Ultraviolet solar radiation	Infra red radiation	Visible light	Gamma rays	Ultra violet solar radiation
52	Which gas has greatest effect on global warming?	Ethane	Methane	CO_2	Hydrogen	Methane
53	The major pollutant from automobile exhaust is ________	NO_2	CO	SO_2	Soot	CO
54	Optimum rate of relative humidity for the survival of most microorganisms is between	10-20%	30-60%	40-80 %	80-100%	40-80 %
55	Human impact on the environment is termed as	Metagenic	Mutagenic	Carcinogenic	Anthropogenic	Anthropogenic

56	Percentage of oxygen is atmospheric air	40.9	60.9	75.9	20.9	20.9
57	Which gas dominates composition of air?	CO2	Oxygen	Nitrogen	Hydrogen	Nitrogen
58	Air pollution is severe in ____________	Cities	Agricultural field	Schools	Desert	Cities
59	Identify the term that describes an environment completely free of microorganisms	Antibiotic	Asepsis	Antisepsis	Probiotic	Asepsis
60	Fine organic and inorganic particles suspended in air is called __________	Particulate pollutant	gaseous pollutant	aerosol	none of these	aerosol

Unit III

Bioremediation

Bioremediation – contaminated soil, aquifers, marine pollutants, air pollutants, stimulation of oil spills degradation. Bioremediation of air pollutants. Bioleaching – recovery of metal from ores – oxidation of minerals – testing for biodegradability

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. Technologies can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site, while *ex siti* involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies, phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration and biostimulation. Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth (i.e., bioavailability) of the pollution-eating microbes within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators. However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil

Bone char has been shown to bioremediate small amounts of Cadmium, Copper, and Zinc. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. Some damaged artifacts at museums contain microbes which could be specified as bio remediating agents. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes. The use of genetic engineering to create organisms specifically designed for bioremediation has great potential. The bacterium *Deinococcus radiodurans* (the most radio resistant organism known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste.

Mycoremediation : Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The term *mycoremediation* refers specifically to the use of fungal mycelia in bioremediation. One of the primary roles of fungi in

the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have been reported to successfully degrade the nerve gases VX and sarin. In one conducted experiment, a plot of soil contaminated with diesel oil was inoculated with mycelia of oyster mushrooms; traditional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95% of many of the PAH (polycyclic aromatic hydrocarbons) had been reduced to non-toxic components in the mycelial-inoculated plots. It appears that the natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds (certain persistent pesticides; Battelle, 2000). Two species of the Ecuadorian fungus Pestalotiopsis are capable of consuming Polyurethane in aerobic and anaerobic conditions such as found at the bottom of landfills.

Mycofiltration is a similar process, using fungal mycelia to filter toxic waste and microorganisms from water in soil. Advantages - There are a number of cost/efficiency advantages to bioremediation, which can be employed in areas that are inaccessible without excavation. For example, hydrocarbon spills (specifically, petrolspills) or certain chlorinated solvents may contaminate groundwater, and introducing the appropriate electron acceptor or electron donor amendment, as appropriate, may significantly reduce contaminant concentrations after a long time allowing for acclimation. This is typically much less expensive than excavation followed by disposal elsewhere, incineration or other *ex situ* treatment strategies, and reduces or eliminates the need for "pump and treat", a practice common at sites where hydrocarbons have contaminated clean groundwater.

Monitoring bioremediation: The process of bioremediation can be monitored indirectly by measuring the Oxidation/ Reduction potential or redox in soil and ground water together with pH, temperature, oxygen content, electron acceptor/donor concentrations, and concentration of breakdown products (e.g. carbon dioxide). This table shows the (decreasing) biological breakdown rate as function of the redox potential.

Process	**Reaction**	**Redox potential (E_h in mV)**
aerobic:	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	600 ~ 400

anaerobic:		
denitrification	$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$	500 ~ 200
manganese IV reduction	$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O$	400 ~ 200
iron III reduction	$Fe(OH)_3 + e^- + 3H^+ \rightarrow Fe^{2+} + 3H_2O$	300 ~ 100
sulfate reduction	$SO_4^{2-} + 8e^- + 10\ H^+ \rightarrow H_2S + 4H_2O$	0 ~ −150
fermentation	$2CH_2O \rightarrow CO_2 + CH_4$	−150 ~ −220

This, by itself and at a single site, gives little information about the process of remediation. It is necessary to sample enough points on and around the contaminated site to be able to determine contours of equal redox potential. Contouring is usually done using specialized software, e.g. using Kriging interpolation. If all the measurements of redox potential show that electron acceptors have been used up, it is in effect an indicator for total microbial activity. Chemical analysis is also required to determine when the levels of contaminants and their breakdown products have been reduced to below regulatory limits.

Soil contamination: Soil contamination or soil pollution is caused by the presence of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons, polynuclear aromatic hydrocarbons (such as naphthalene and benzo (a) pyrene) solvents, pesticides, lead, and other heavy metals. Contamination is correlated with the degree of industrialization and intensity of chemical usage. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapors from the contaminants, and from secondary contamination of water supplies within and underlying the soil. Mapping of contaminated soil sites and the resulting cleanup are time consuming and expensive tasks, requiring extensive amounts of geology, hydrology, chemistry, computer modeling skills, and GIS in Environmental Contamination, as well as an appreciation of the history of industrial chemistry. In North America and Western Europe that the extent of contaminated land is best known, with many of countries in these areas having a legal framework to identify and deal with this environmental problem. Developing countries tend to be less tightly regulated despite some of them having undergone significant industrialization.

Causes: Soil pollution can be caused by: Application of pesticides, herbicides and fertilizers - Mining, Oil and fuel dumping, Disposal of coal ash, Leaching from landfills, Drainage of contaminated surface water into the soil, Discharging urine and feces in the open. Electronic waste, The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, lead, and other heavy metals.

Coal ash: Historical deposition of coal ash used for residential, commercial, and industrial heating, as well as for industrial processes such as ore smelting, were a common source of contamination in areas that were industrialized before about 1960. Coal naturally concentrates lead and zinc during its formation, as well as other heavy metals to a lesser degree. When the coal is burned, most of these metals become concentrated in the ash (the principal exception being mercury). Coal ash and slag may contain sufficient lead to qualify as a "characteristic hazardous waste", defined in the USA as containing more than 5 mg/L of extractable lead using the TCLP procedure. In addition to lead, coal ash typically contains variable but significant concentrations of polynuclear aromatic hydrocarbons (PAHs; e.g., benzo (a) anthracene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, indeno (cd) pyrene, phenanthrene, anthracene, and others). These PAHs are known human carcinogens and the acceptable concentrations of them in soil are typically around 1 mg/kg. Coal ash and slag can be recognized by the presence of off-white grains in soil, gray heterogeneous soil, or (coal slag) bubbly, vesicular pebble-sized grains.jk

Sewage: Treated sewage sludge, known in the industry as biosolids, has become controversial as a fertilizer to the land. As it is the byproduct of sewage treatment, it generally contains more contaminants such as organisms, pesticides, and heavy metals than other soil. In the European Union, the Urban Waste Water Treatment Directive allows sewage sludge to be sprayed onto land. The volume is expected to double to 185,000 tons of dry solids in 2005. This has good agricultural properties due to the high nitrogen and phosphate content. In 1990/1991, 13% wet weight was sprayed onto 0.13% of the land; however, this is expected to rise 15 fold by 2005. Advocates say there is a need to control this so that pathogenic microorganisms do not get into water courses and to ensure that there is no accumulation of heavy metals in the top soil.

Pesticides and herbicides: A pesticide is a substance or mixture of substances used to kill a pest. A pesticide may be a chemical substance, biological agent (such as a virus or bacteria), antimicrobial, disinfectant or device used against any pest. Pests include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread or are a vector for disease or cause a nuisance. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other organisms. Herbicides are used to kill weeds, especially on pavements and railways. They are similar to auxins and most are biodegradable by soil bacteria. However, one group derived from trinitrotoluene(2:4 D and 2:4:5 T) have the impurity dioxin, which is very toxic and causes fatality even in low concentrations. Another herbicide is Paraquat. It is highly toxic but it rapidly degrades in soil due to the action of bacteria and does not kill soil fauna. Insecticides are used to rid farms of pests which damage crops. The insects damage not only standing crops but also stored ones and in the tropics it is reckoned that one third of the total production is lost during food storage. As with fungicides, the first insecticides used in the nineteenth century were inorganic e.g. Paris green and other compounds of arsenic. Nicotine has also been used since the late eighteenth century.

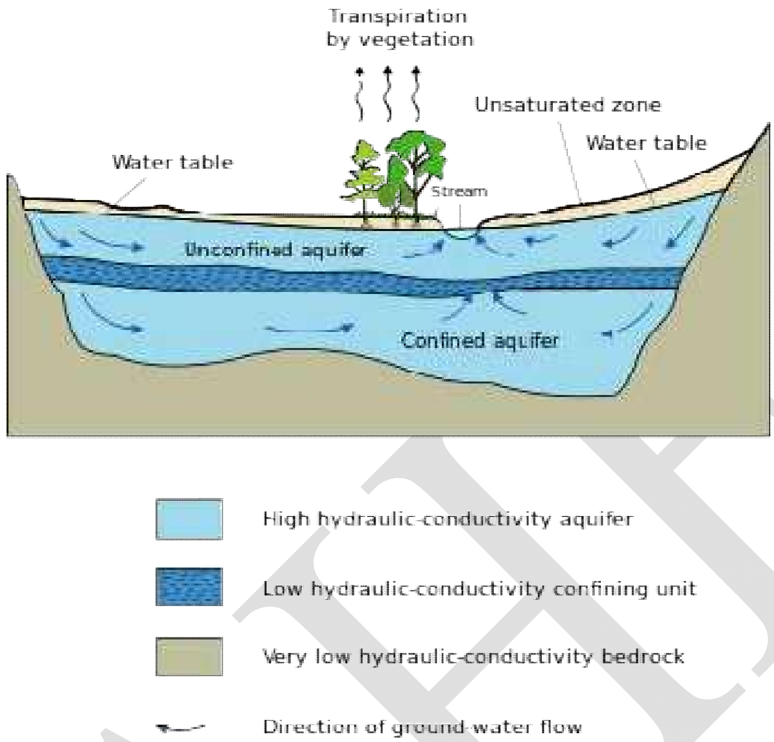
There are now two main groups of synthetic insecticides: Organochlorines include DDT, Aldrin, Dieldrin and BHC. They are cheap to produce, potent and persistent. DDT was used on a massive scale from the 1930s, with a peak of 72,000 tones used 1970. Then usage fell as the harmful environmental effects were realized. It was found worldwide in fish and birds and was even discovered in the snow in the Antarctic. It is only slightly soluble in water but is very soluble in the bloodstream. It affects the nervous and endocrine systems and causes the eggshells of birds to lack calcium causing them to be easily breakable. It is thought to be responsible for the decline of the numbers of birds of prey like ospreys and peregrine falcons in the 1950s - they are now recovering. As well as increased concentration via the food chain, it is known to enter via permeable membranes, so fish get it through their gills. As it has low water solubility, it tends to stay at the water surface, so organisms that live there are most affected. DDT found in fish that formed part of the human food chain caused concern, but the levels found in the liver, kidney and brain tissues was less than 1 ppm and in fat was 10 ppm which was below the level likely to cause harm. However, DDT was banned in the UK and the United States to stop the further build up of it in the food chain. U.S. manufacturers continued to sell DDT to developing countries, who could not afford the expensive replacement chemicals and who did not have such stringent regulations governing the use of pesticides.

Health effects: Contaminated or polluted soil directly affects human health through direct contact with soil or via inhalation of soil contaminants which have vaporized; potentially greater threats are posed by the infiltration of soil contamination into groundwater aquifers used for human consumption, sometimes in areas apparently far removed from any apparent source of above ground contamination. Health consequences from exposure to soil contamination vary greatly depending on pollutant type, pathway of attack and vulnerability of the exposed population. Chronic exposure to chromium, lead and other metals, petroleum, solvents, and many pesticide and herbicide formulations can be carcinogenic, can cause congenital disorders, or can cause other chronic health conditions. Industrial or man-made concentrations of naturally occurring substances, such as nitrate and ammonia associated with livestock manure from agricultural operations, have also been identified as health hazards in soil and groundwater. Chronic exposure to benzene at sufficient concentrations is known to be associated with higher incidence of leukemia. Mercury and cyclodienes are known to induce higher incidences of kidney damage, some irreversible. PCBs and cyclodienes are linked to liver toxicity. Organophosphates and carbomates can induce a chain of responses leading to neuromuscular blockage. Many chlorinated solvents induce liver changes, kidney changes and depression of the central nervous system. There is an entire spectrum of further health effects such as headache, nausea, fatigue, eye irritation and skin rash for the above cited and other chemicals. At sufficient dosages a large number of soil contaminants can cause death by exposure via direct contact, inhalation or ingestion of contaminants in groundwater contaminated through soil. The Scottish Government has commissioned the Institute of Occupational Medicine to undertake a review of methods to assess risk to human health from contaminated land. The overall aim of the project is to work up guidance that should be useful to Scottish Local Authorities in assessing whether sites represent a significant possibility of significant harm (SPOSH) to human health. It is envisaged that the output of the project will be a short document providing high level guidance on health risk assessment with reference to existing published guidance and methodologies that have been identified as being particularly relevant and helpful. The project will examine how policy guidelines have been developed for determining the acceptability of risks to human health and propose an approach for assessing what constitutes unacceptable risk in line with the criteria for SPOSH as defined in the legislation and the Scottish Statutory Guidance.

Ecosystem effects: Not unexpectedly, soil contaminants can have significant deleterious consequences for ecosystems. There are radical soil chemistry changes which can arise from the presence of many hazardous chemicals even at low concentration of the contaminant species. These changes can manifest in the alteration of metabolism of endemic microorganisms and arthropods resident in a given soil environment. The result can be virtual eradication of some of the primary food chain, which in turn could have major consequences for predator or consumer species. Even if the chemical effect on lower life forms is small, the lower pyramid levels of the food chain may ingest alien chemicals, which normally become more concentrated for each consuming rung of the food chain. Many of these effects are now well known, such as the concentration of persistent DDT materials for avian consumers, leading to weakening of egg shells, increased chick mortality and potential extinction of species. Effects occur to agricultural lands which have certain types of soil contamination. Contaminants typically alter plant metabolism, often causing a reduction in crop yields. This has a secondary effect upon soil conservation, since the languishing crops cannot shield the Earth's soil from erosion. Some of these chemical contaminants have long half-lives and in other cases derivative chemicals are formed from decay of primary soil contaminants.

Cleanup options: Clean up or environmental remediation is analyzed by environmental scientists who utilize field measurement of soil chemicals and also apply computer models(GIS in Environmental Contamination) for analyzing transport and fate of soil chemicals. There are several principal strategies for remediation: Excavate soil and take it to a disposal site away from ready pathways for human or sensitive ecosystem contact. This technique also applies to dredging of bay muds containing toxins. Aeration of soils at the contaminated site (with attendant risk of creating air pollution. Thermal remediation by introduction of heat to raise subsurface temperatures sufficiently high to volatize chemical contaminants out of the soil for vapour extraction. Technologies include ISTD, electrical resistance heating (ERH), and ET-DSP^{tm}. Bioremediation, involving microbial digestion of certain organic chemicals. Techniques used in bioremediation include landfarming, biostimulation and bioaugmentating soil biota with commercially available microflora. Extraction of ground water or soil vapor with an active electromechanical system, with subsequent stripping of the contaminants from the extract. Containment of the soil contaminants (such as by capping or paving over in place). Phytoremediation, or using plants (such as willow) to extract heavy metals.

Aquifer: An aquifer is an underground layer of water-bearing permeable rock or unconsolidated materials (gravel, sand, or silt) from which ground water can be extracted using a water well. The study of water flow in aquifers and the characterization of aquifers is called hydrogeology. Related terms include aquitard, which is a bed of low permeability along an aquifer, and aquiclude (or*aquifuge*), which is a solid, impermeable area underlying or overlying an aquifer. If the impermeable area overlies the aquifer pressure could cause it to become a confined aquifer.



Depth: Aquifers may occur at various depths. Those closer to the surface are not only more likely to be used for water supply and irrigation, but are also more likely to be topped up by the local rainfall. Many desert areas have limestone hills or mountains within them or close to them that can be exploited as groundwater resources. Parts of the Atlas Mountains in North Africa, the Lebanon and Anti-Lebanon ranges of Syria, Israel and Lebanon, the Jebel Akhdar (Oman) in Oman, parts of the Sierra Nevada and neighboring ranges in the United States' Southwest, have shallow aquifers that are exploited for their water. Over-exploitation can lead to the exceeding of the practical sustained yield; i.e., more water is taken out than can be replenished. Along the coastlines of certain countries, such as Libya and Israel, population growth has led to overpopulation, which has caused the lowering of water table and the subsequent contamination of the groundwater with saltwater from the sea. Many of these withdrawals are caused by farmers and industrial plants adding pollutants such as fertilizers and chemical waste to the aquifer's water supply, which quickly dry up the water and contaminate the soil. The beach provides a model to help visualize an aquifer. If a hole is dug into the sand, very wet or saturated sand will be located at a shallow depth. This hole is a crude well, the wet sand represents an aquifer, and the level to which the water rises in this hole represents the water table.

Classification: The above diagram indicates typical flow directions in a cross-sectional view of a simple confined or unconfined aquifer system. The system shows two aquifers with one aquitard (a confining or impermeable layer) between them, surrounded by the bedrock *aquiclude*, which is in contact with a gaining stream (typical in humid regions). The water table and unsaturated zone are also illustrated. An *aquitard* is a zone within the earth that restricts the flow of groundwater from one aquifer to another. An aquitard can sometimes, if completely impermeable, be called an *aquiclude* or *aquifuge*. Aquitards are composed of layers of either clay or non-porous rock with low hydraulic conductivity.

Saturated versus unsaturated: Groundwater can be found at nearly every point in the Earth's shallow subsurface, to some degree; although aquifers do not necessarily contain fresh water. The Earth's crust can be divided into two regions: the *saturated zone* or *phreatic zone* (e.g., aquifers, aquitards, etc.), where all available spaces are filled with water, and the *unsaturated zone* (also called the vadose zone), where there are still pockets of air that contain some water, but can be filled with more water.Saturated means the pressure head of the water is greater than atmospheric pressure (it has a gauge pressure > 0). The definition of the water table is surface where the pressure head is equal to atmospheric pressure (where gauge pressure = 0). Unsaturated conditions occur above the water table where the pressure head is negative (absolute pressure can never be negative, but gauge pressure can) and the water that incompletely fills the pores of the aquifer material is under suction. The water content in the unsaturated zone is held in place by surface adhesive forces and it rises above the water table (the zero gauge pressure isobar by capillary action to saturate a small zone above the phreatic surface (the capillary fringe) at less than atmospheric pressure. This is termed tension saturation and is not the same as saturation on a water content basis. Water content in a capillary fringe decreases with increasing distance from the phreatic surface. The capillary head depends on soil pore size. In sandy soils with larger pores, the head will be less than in clay soils with very small pores. The normal capillary rise in a clayey soil is less than 1.80 m (six feet) but can range between 0.3 and 10 m (1 and 30 ft). The capillary rise of water in a small diameter tube is this same physical process. The water table is the level to which water will rise in a large-diameter pipe (e.g., a well) that goes down into the aquifer and is open to the atmosphere.

Basal aquifer: Basal aquifer or basal water sands aquifers (BWS) describes "the water-bearing sands, gravel or fractured rock that is found at the bottom of a geological formation, underlying the bitumen-saturated sands. An example is the McMurray basal water sands aquifer. The McMurray Formation in the Athabaska oil sands in northern Alberta, consists of "sandstone and shale deposited in a transgressive geological sequence", resulting in the "course grained texture of the basal deposits". Basal water sands (BWS) aquifers occur when the basal sand is low in bitumen. Although the water at this depth may be saline, portions of the water-saturated McMurray basal water sand aquifer are non-saline (FMFN & 2012 31,105).

Aquifers versus aquitards: Aquifers are typically saturated regions of the subsurface that produce an economically feasible quantity of water to a well or spring (e.g., sand and gravel or fractured bedrock often make good aquifer materials). An aquitard is a zone within the earth that restricts the flow of groundwater from one aquifer to another. An aquitard can sometimes, if completely impermeable, be called an aquiclude oraquifuge. Aquitards comprise layers of either clay or non-porous rock with low hydraulic conductivity. In mountainous areas (or near rivers in mountainous areas), the main aquifers are typically unconsolidated alluvium, composed of mostly horizontal layers of materials deposited by water processes (rivers and streams), which in cross-section (looking at a two-dimensional slice of the aquifer) appear to be layers of alternating coarse and fine materials. Coarse materials, because of the high energy needed to move them, tend to be found nearer the source (mountain fronts or rivers), whereas the fine-grained material will make it farther from the source (to the flatter parts of the basin or overbank areas - sometimes called the pressure area). Since there are less fine-grained deposits near the source, this is a place where aquifers are often unconfined (sometimes called the fore bay area), or in hydraulic communication with the land surface.

Confined versus unconfined: There are two end members in the spectrum of types of aquifers; *confined* and *unconfined* (with semi-confined being in between). Unconfined aquifers are sometimes also called *water table* or *phreatic* aquifers, because their upper boundary is the water table or phreatic surface. Typically (but not always) the shallowest aquifer at a given location is unconfined, meaning it does not have a confining layer (an aquitard or aquiclude) between it and the surface. The term "perched" refers to ground water accumulating above a low-permeability unit or strata, such as a clay layer. This term is generally used to refer to a small local area of ground water that occurs at an elevation higher than a regionally extensive aquifer. The difference between perched and unconfined aquifers is their size (perched is smaller). If the distinction between confined and unconfined is not clear geologically (i.e., if it is not known if a clear confining layer exists, or if the geology is more complex, e.g., a fractured bedrock aquifer), the value of storativity returned from an aquifer test can be used to determine it (although aquifer tests in unconfined aquifers should be interpreted differently than confined ones). Confined aquifers have very low storativity values (much less than 0.01, and as little as 10^{-5}), which means that the aquifer is storing water using the mechanisms of aquifer matrix expansion and the compressibility of water, which typically are both quite small quantities. Unconfined aquifers have storativities (typically then called specific yield) greater than 0.01 (1% of bulk volume); they release water from storage by the mechanism of actually draining the pores of the aquifer, releasing relatively large amounts of water (up to the drainable porosity of the aquifer material, or the minimum volumetric water content). Isotropic versus anisotropic In isotropic aquifers or aquifer layers the hydraulic conductivity (K) is equal for flow in all directions, while in anisotropic conditions it differs, notably in horizontal (Kh) and vertical (Kv) sense. Semi-confined aquifers with one or more aquitards work as an anisotropic system, even when the separate layers are isotropic, because the compound Kh and Kv values are different (seehydraulic transmissivity and hydraulic resistance). When calculating flow to drains or flow to wells in an aquifer, the anisotropy is to be taken into account lest the resulting design of the drainage system may be faulty.

Groundwater in rock formations: Groundwater may exist in *underground rivers* (e.g., caves where water flows freely underground). This may occur in eroded limestone areas known as karst topography, which make up only a small percentage of Earth's area. More usual is that the pore spaces of rocks in the subsurface are simply saturated with water like a kitchen sponge which can be pumped out for agricultural, industrial, or municipal uses. If a rock unit of low porosity is highly fractured, it can also make a good aquifer (via fissure flow), provided the rock has a hydraulic conductivity sufficient to facilitate movement of water. Porosity is important, but, *alone*, it does not determine a rock's ability to act as an aquifer. Areas of the Deccan Traps (basaltic lava) in west central India are good examples of rock formations with high porosity but low permeability, which makes them poor aquifers. Similarly, the micro-porous (Upper Cretaceous) Chalk of south east England, although having a reasonably high porosity, has a low grain-to-grain permeability, with its good water-yielding characteristics mostly due to micro-fracturing and fissuring.

Human dependence on groundwater: Most land areas on Earth have some form of aquifer underlying them, sometimes at significant depths. These aquifers are rapidly being depleted by the human population. Fresh-water aquifers, especially those with limited recharge by meteoric water, can be over-exploited and, depending on the local hydrogeology, may draw in non-potable water or saltwater intrusion from hydraulically connected aquifers or surface water bodies. This can be a serious problem, especially in coastal areas and other areas where aquifer pumping is excessive. In some areas, the ground water can be contaminated by mineral poisons, such as arsenic -see Arsenic contamination of groundwater. Aquifers are critically important in human habitation and agriculture. Deep aquifers in arid areas have long been water sources for irrigation. Many villages and even large cities draw their water supply from wells in aquifers.

Municipal, irrigation, and industrial water supplies are provided through large wells. Multiple wells for one water supply source are termed "wellfields", which may withdraw water from confined or unconfined aquifers. Using ground water from deep, confined aquifers provides more protection from surface water contamination. Some wells, termed "collector wells," are specifically designed to induce infiltration of surface (usually river) water. Aquifers that provide sustainable fresh groundwater to urban areas and for agricultural irrigation are typically close to the ground surface (within a couple of hundred metres) and have some recharge by fresh water. This recharge is typically from rivers or meteoric water (precipitation) that percolates into the aquifer through overlying unsaturated materials. Occasionally, sedimentary or "fossil" aquifers are used to provide irrigation and drinking water to urban areas. In Libya, for example, Muammar Gaddafi's Great Manmade River project has pumped large amounts of groundwater from aquifers beneath the Sahara to populous areas near the coast. Though this has saved Libya money over the alternative, desalination, the aquifers are likely to run dry in 60 to 100 years. Aquifer depletion has been cited as one of the causes of the food price rises of 2011.

Subsidence: In unconsolidated aquifers, groundwater is produced from pore spaces between particles of gravel, sand, and silt. If the aquifer is confined by low-permeability layers, the reduced water pressure in the sand and gravel causes slow drainage of water from the adjoining confining layers. If these confining layers are composed of compressible silt or clay, the loss of water to the aquifer reduces the water pressure in the confining layer, causing it to compress from the weight of overlying geologic materials. In severe cases, this compression can be observed on the ground surface as subsidence. Unfortunately, much of the subsidence from groundwater extraction is permanent (elastic rebound is small). Thus, the subsidence is not only permanent, but the compressed aquifer has a permanently reduced capacity to hold water.

Saltwater intrusion: Aquifers near the coast have a lens of freshwater near the surface and denser seawater under freshwater. Seawater penetrates the aquifer diffusing in from the ocean and is denser than freshwater. For porous aquifers near the coast, the thickness of freshwater atop saltwater is about 40 feet (12 m) for every 1 ft (0.30 m) of freshwater head above sea level. This relationship is called the Ghyben-Herzberg equation. If too much ground water is pumped near the coast, salt-water may intrude into freshwater aquifers causing contamination of potable freshwater supplies. Many coastal aquifers, such as the Biscayne Aquifer near Miami and the New Jersey Coastal Plain aquifer, have problems with saltwater intrusion as a result of over pumping.

Salination: Aquifers in surface irrigated areas in semi-arid zones with reuse of the unavoidable irrigation water losses percolating down into the underground by supplemental irrigation from wells run the risk of salination. Surface irrigation water normally contains salts in the order of

g/l or more and the annual irrigation requirement is in the order of 10000 m^3/ha or more so the annual import of salt is in the order of 5000 kg/ha or more. Under the influence of continuous evaporation, the salt concentration of the aquifer water may increase continually and eventually cause anenvironmental problem. For salinity control in such a case, annually an amount of drainage water is to be discharged from the aquifer by means of a subsurface drainage system and disposed of through a safe outlet. The drainage system may be *horizontal* (i.e. using pipes, tile drains or ditches) or *vertical* (drainage by wells). To estimate the drainage requirement, the use of a groundwater model with an agro-hydro-salinity component may be instrumental, e.g. Sahys Mod.

Examples: The Great Artesian Basin situated in Australia is arguably the largest groundwater aquifer in the world (over 1.7 million km^2). It plays a large part in water supplies for Queensland and remote parts of South Australia. The Guarani Aquifer, located beneath the surface of Argentina, Brazil, Paraguay, and Uruguay, is one of the world's largest aquifer systems and is an important source of fresh water. Named after the Guarani people, it covers 1,200,000 km^2, with a volume of about 40,000 km^3, a thickness of between 50 m and 800 m and a maximum depth of about 1,800 m. Aquifer depletion is a problem in some areas, and is especially critical in northern Africa; see the Great Manmade River project of Libya for an example. However, new methods of groundwater management such as artificial recharge and injection of surface waters during seasonal wet periods has extended the life of many freshwater aquifers, especially in the United States. The Ogallala Aquifer of the central United States is one of the world's great aquifers, but in places it is being rapidly depleted by growing municipal use, and continuing agricultural use. This huge aquifer, which underlies portions of eight states, contains primarily fossil water from the time of the last glaciation. Annual recharge, in the more arid parts of the aquifer, is estimated to total only about 10 percent of annual withdrawals. According to a 2013 report by research hydrologist, Leonard F. Konikow, at the United States Geological Survey (USGC), the depletion between 2001–2008, inclusive, is about 32 percent of the cumulative depletion during the entire 20th century (Konikow 2013:22). In the United States, the biggest users of water from aquifers include agricultural irrigation and oil and coal extraction. Cumulative total groundwater depletion in the United States accelerated in the late 1940s and continued at an almost steady linear rate through the end of the century. In addition to widely recognized environmental consequences, groundwater depletion also adversely impacts the long-term sustainability of groundwater supplies to help meet the Nation’s water needs. An example of a significant and sustainable carbonate aquifer is the Edwards Aquifer in central Texas. This carbonate aquifer has historically been providing high quality water for nearly 2 million people, and even today, is full because of tremendous recharge from a number of area streams, rivers and lakes. The primary risk to this resource is human development over the recharge areas.

Marine pollution: Marine pollution occurs when harmful, or potentially harmful, effects result from the entry into the ocean of chemicals, particles, industrial, agricultural and residential waste, noise, or the spread of invasive organisms. Most sources of marine pollution are land based. The pollution often comes from nonpoint sources such as agricultural runoff, wind-blown debris and dust. Nutrient pollution, a form of water pollution, refers to contamination by excessive inputs of nutrients. It is a primary cause of eutrophication of surface waters, in which excess nutrients, usually nitrogen or phosphorus, stimulate algal growth. Many potentially toxic chemicals adhere to tiny particles which are then taken up by plankton and benthos animals, most of which are either deposit or filter feeders. In this way, the toxins are concentrated upward within ocean food chains. Many particles combine chemically in a manner highly depletive of oxygen, causing estuaries to become anoxic. When pesticides are incorporated into the marine ecosystem, they quickly become absorbed into marine food webs. Once in the food webs, these pesticides can cause mutations, as well as diseases, which can be harmful to humans as well as the entire food web. Toxic metals can also be introduced into marine food webs. These can cause a change to tissue matter, biochemistry, behaviour, reproduction, and suppress growth in marine life. Also, many animal feeds have a high fish meal or fish hydrolysate content. In this way, marine toxins can be transferred to land animals, and appear later in meat and dairy products

History: Although marine pollution has a long history, significant international laws to counter it were only enacted in the twentieth century. Marine pollution was a concern during several United Nations Conferences on the Law of the Sea beginning in the 1950s. Most scientists believed that the oceans were so vast that they had unlimited ability to dilute, and thus render pollution, harmless. In the late 1950s and early 1960s, there were several controversies about dumping radioactive waste off the coasts of the United States by companies licensed by the Atomic Energy Commission, into the Irish Sea from the British reprocessing facility at Windscale, and into the Mediterranean Sea by the French Commissariat à l'Energie Atomique. After the Mediterranean Sea controversy, for example, Jacques Cousteau became a worldwide figure in the campaign to stop marine pollution. Marine pollution made further international headlines after the 1967 crash of the oil tanker Torrey Canyon, and after the 1969 Santa Barbara oil spill off the coast of California. Marine pollution was a major area of discussion during the 1972 United Nations Conference on the Human Environment, held in Stockholm. That year also saw the signing of the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, sometimes called the London Convention. The London Convention did not ban marine pollution, but it established black and gray lists for substances to be banned (black) or regulated by national authorities (gray). Cyanide and high-level radioactive waste, for example, were put on the black list. The London Convention applied only to waste dumped from ships, and thus did nothing to regulate waste discharged as liquids from pipelines.

Pathways of pollution: There are many different ways to categorize, and examine the inputs of pollution into our marine ecosystems. Patin notes that generally there are three main types of inputs of pollution into the ocean: direct discharge of waste into the oceans, runoff into the waters due to rain, and pollutants that are released from the atmosphere. One common path of entry by contaminants to the sea are rivers. The evaporation of water from oceans exceeds precipitation. The balance is restored by rain over the continents entering rivers and then being returned to the sea. The Hudson in New York State and the Raritan in New Jersey, which empty at the northern and southern ends of Staten Island, are a source of mercury contamination of zooplankton (copepods) in the open ocean. The highest concentration in the filter-feeding copepods is not at the mouths of these rivers but 70 miles south, nearer Atlantic City, because water flows close to the coast. It takes a few days before toxins are taken up by the plankton. Pollution is often classed as point source or nonpoint source pollution. Point source pollution occurs when there is a single, identifiable, and localized source of the pollution. An example is directly discharging sewage and industrial waste into the ocean. Pollution such as this occurs particularly in developing nations.

Direct discharge: Pollutants enter rivers and the sea directly from urban sewerage and industrial waste discharges, sometimes in the form of hazardous and toxic wastes. Inland mining for copper, gold. etc., is another source of marine pollution. Most of the pollution is simply soil, which ends up in rivers flowing to the sea. However, some minerals discharged in the course of the mining can cause problems, such as copper, a common industrial pollutant, which can interfere with the life history and development of coral polyps. Mining has a poor environmental track record. For example, according to the United States Environmental Protection Agency, mining has contaminated portions of the headwaters of over 40% of watersheds in the western continental US. Much of this pollution finishes up in the sea.

Land runoff: Surface runoff from farming, as well as urban runoff and runoff from the construction of roads, buildings, ports, channels, and harbours, can carry soil and particles laden with carbon, nitrogen, phosphorus, and minerals. This nutrient-rich water can cause fleshy algae and phytoplankton to thrive in coastal areas; known as algal blooms, which have the potential to create hypoxic conditions by using all available oxygen. Polluted runoff from roads and highways can be a significant source of water pollution in coastal areas. About 75 percent of the toxic chemicals that flow into Puget Sound are carried by storm water that runs off paved roads and driveways, rooftops, yards and other developed land.

Ship pollution: Ships can pollute waterways and oceans in many ways. Oil spills can have devastating effects. While being toxic to marine life, polycyclic aromatic hydrocarbons (PAHs), found in crude oil, are very difficult to clean up, and last for years in the sediment and marine environment. Discharge of cargo residues from bulk carriers can pollute ports, waterways and oceans. In many instances vessels intentionally discharge illegal wastes despite foreign and domestic regulation prohibiting such actions. It has been estimated that container ships lose over 10,000 containers at sea each year (usually during storms). Ships also create noise pollution that disturbs natural wildlife, and water from ballast tanks can spread harmful algae and other invasive species.

Ballast water taken up at sea and released in port is a major source of unwanted exotic marine life. The invasive freshwater zebra mussels, native to the Black, Caspian and Azov seas, were probably transported to the Great Lakes via ballast water from a transoceanic vessel. Meinesz believes that one of the worst cases of a single invasive species causing harm to an ecosystem can be attributed to a seemingly harmless jellyfish. *Mnemiopsis leidyi*, a species of comb jellyfish that spread so it now inhabits estuaries in many parts of the world. It was first introduced in 1982, and thought to have been transported to the Black Sea in a ship's ballast water. The population of the jellyfish shot up exponentially and, by 1988, it was wreaking havoc upon the local fishing industry. ―The anchovy catch fell from 204,000 tons in 1984 to 200 tons in 1993; sprat from 24,600 tons in 1984 to 12,000 tons in 1993; horse mackerel from 4,000 tons in 1984 to zero in 1993.‖ Now that the jellyfish have exhausted the zooplankton, including fish larvae, their numbers have fallen dramatically, yet they continue to maintain a stranglehold on the ecosystem. Invasive species can take over once occupied areas, facilitate the spread of new diseases, introduce new genetic material, alter underwater seascapes and jeopardize the ability of native species to obtain food. Invasive species are responsible for about $138 billion annually in lost revenue and management costs in the US alone.

Atmospheric pollution: Another pathway of pollution occurs through the atmosphere. Windblown dust and debris, including plastic bags, are blown seaward from landfills and other areas. Dust from the Sahara moving around the southern periphery of the subtropical ridge moves into the Caribbean and Florida during the warm season as the ridge builds and moves northward through the subtropical Atlantic. Dust can also be attributed to a global transport from the Gobi and Taklamakan deserts across Korea, Japan, and the Northern Pacific to the Hawaiian Islands. Since 1970, dust outbreaks have worsened due to periods of drought in Africa. There is a large variability in dust transport to the Caribbean and Florida from year to year; however, the flux is greater during positive phases of the North Atlantic Oscillation. The USGS links dust events to a decline in the health of coral reefs across the Caribbean and Florida, primarily since the 1970s. Climate change is raising ocean temperatures and raising levels of carbon dioxide in the atmosphere. These rising levels of carbon dioxide are acidifying the oceans. This, in turn, is altering aquatic ecosystems and modifying fish distributions, with impacts on the sustainability of fisheries and the livelihoods of the communities that depend on them. Healthy ocean ecosystems are also important for the mitigation of climate change.

Deep sea mining: Deep sea mining is a relatively new mineral retrieval process that takes place on the ocean floor. Ocean mining sites are usually around large areas of poly metallic nodules or active and extinct hydrothermal vents at about 1,400 - 3,700 meters below the ocean’s surface. The vents create sulfide deposits, which contain precious metals such as silver, gold, copper, manganese, cobalt, and zinc. The deposits are mined using either hydraulic pumps or bucket systems that take ore to the surface to be processed. As with all mining operations, deep sea mining raises questions about environmental damages to the surrounding areas. Because deep sea mining is a relatively new field, the complete consequences of full scale mining operations are unknown. However, experts are certain that removal of parts of the sea floor will result in disturbances to the benthic layer, increased toxicity of the water column and sediment plumes from tailings.

Removing parts of the sea floor disturbs the habitat of benthic organisms, possibly, depending on the type of mining and location, causing permanent disturbances. Aside from direct impact of mining the area, leakage, spills and corrosion would alter the mining area’s chemical makeup. Among the impacts of deep sea mining, sediment plumes could have the greatest impact. Plumes are caused when the tailings from mining (usually fine particles) are dumped back into the ocean, creating a cloud of particles floating in the water. Two types of plumes occur: near bottom plumes and surface plumes. Near bottom plumes occur when the tailings are pumped back down to the mining site. The floating particles increase the turbidity, or cloudiness, of the water, clogging filter-feeding apparatuses used by benthic organisms. Surface plumes cause a more serious problem. Depending on the size of the particles and water currents the plumes could spread over vast areas. The plumes could impact zooplankton and light penetration, in turn affecting the food web of the area.

Types of pollution

Acidification: The oceans are normally a natural carbon sink, absorbing carbon dioxide from the atmosphere. Because the levels of atmospheric carbon dioxide are increasing, the oceans are becoming more acidic. The potential consequences of ocean acidification are not fully understood, but there are concerns that structures made of calcium carbonate may become vulnerable to dissolution, affecting corals and the ability of shellfish to form shells. Oceans and coastal ecosystems play an important role in the global carbon cycle and have removed about 25% of the carbon dioxide emitted by human activities between 2000 and 2007 and about half the anthropogenic CO_2 released since the start of the industrial revolution. Rising ocean temperatures and ocean acidification means that the capacity of the ocean carbon sink will gradually get weaker, giving rise to global concerns expressed in the Monaco and Manado Declarations.

A report from NOAA scientists published in the journal Science in May 2008 found that large amounts of relatively acidified water are upwelling to within four miles of the Pacific continental shelf area of North America. This area is a critical zone where most local marine life lives or is born. While the paper dealt only with areas from Vancouver to northern California, other continental shelf areas may be experiencing similar effects. A related issue is the methane clathrate reservoirs found under sediments on the ocean floors. These trap large amounts of the greenhouse gas methane, which ocean warming has the potential to release. In 2004 the global inventory of ocean methane clathrates was estimated to occupy between one and five million cubic kilo metres. If all these clathrates were to be spread uniformly across the ocean floor, this would translate to a thickness between three and fourteen metres. This estimate corresponds to 500-2500 gigatonnes carbon (Gt C), and can be compared with the 5000 Gt C estimated for all other fossil fuel reserves.

Eutrophication: Eutrophication is an increase in chemical nutrients, typically compounds containing nitrogen or phosphorus, in an ecosystem. It can result in an increase in the ecosystem's primary productivity (excessive plant growth and decay), and further effects including lack of oxygen and severe reductions in water quality, fish, and other animal populations. The biggest culprit are rivers that empty into the ocean, and with it the many chemicals used as fertilizers in agriculture as well as waste from livestock and humans. An excess of oxygen depleting chemicals in the water can lead to hypoxia and the creation of a dead zone. Estuaries tend to be naturally eutrophic because land-derived nutrients are concentrated where runoff enters the marine environment in a confined channel. The World Resources Institute has identified 375 hypoxic coastal zones around the world, concentrated in coastal areas in Western Europe, the Eastern and Southern coasts of the US, and East Asia, particularly in Japan. In the ocean, there are frequent red tide algae blooms that kill fish and marine mammals and cause respiratory problems in humans and some domestic animals when the blooms reach close to shore. In addition to land runoff, atmospheric anthropogenic fixed nitrogen can enter the open ocean. A study in 2008 found that this could account for around one third of the ocean's external (non-recycled) nitrogen supply and up to three per cent of the annual new marine biological production. It has been suggested that accumulating reactive nitrogen in the environment may have consequences as serious as putting carbon dioxide in the atmosphere. One proposed solution to eutrophication in estuaries is to restore shellfish populations, such as oysters. Oyster reefs remove nitrogen from the water column and filter out suspended solids, subsequently reducing the likelihood or extent of harmful algal blooms or anoxic conditions Filter feeding activity is considered beneficial to water quality by controlling phytoplankton density and sequestering nutrients, which can be removed from the system through shellfish harvest, buried in the sediments, or lost through denitrification. Foundational work toward the idea of improving marine water quality through shellfish cultivation to was conducted by Odd Lindahl using mussels in Sweden.

Plastic debris: Marine debris is mainly discarded human rubbish which floats on, or is suspended in the ocean. Eighty percent of marine debris is plastic - a component that has been rapidly accumulating since the end of World War II. The mass of plastic in the oceans may be as high as one hundred million metric tons. Discarded plastic bags, six pack rings and other forms of plastic waste which finish up in the ocean present dangers to wildlife and fisheries. Aquatic life can be threatened through entanglement, suffocation, and ingestion. Fishing nets, usually made of plastic, can be left or lost in the ocean by fishermen. Known as ghost nets, these entangle fish, dolphins, sea turtles, sharks, dugongs, crocodiles, seabirds, crabs, and other creatures, restricting movement, causing starvation, laceration and infection, and, in those that need to return to the surface to breathe, suffocation. Remains of an albatross containing ingested flotsam. Many animals that live on or in the sea consume flotsam by mistake, as it often looks similar to their natural prey. Plastic debris, when bulky or tangled, is difficult to pass, and may become permanently lodged in the digestive tracts of these animals, blocking the passage of food and causing death through starvation or infection. Plastics accumulate because they don't biodegrade in the way many other substances do. They will photo degrade on exposure to the sun, but they do so properly only under dry conditions, and water inhibits this process. In marine environments, photo degraded plastic disintegrates into ever smaller pieces while remaining polymers, even down to the molecular level. When floating plastic particles photo degrade down to zooplankton sizes, jellyfish attempt to consume them, and in this way the plastic enters the ocean food chain. Many of these long-lasting pieces end up in the stomachs of marine birds and animals, including sea turtles, and black-footed albatross.

Plastic debris tends to accumulate at the centre of ocean gyres. In particular, the Great Pacific Garbage Patch has a very high level of plastic particulate suspended in the upper water column. In samples taken in 1999, the mass of plastic exceeded that of zooplankton (the dominant animal life in the area) by a factor of six. Midway Atoll, in common with all the Hawaiian Islands, receives substantial amounts of debris from the garbage patch. Ninety percent plastic, this debris accumulates on the beaches of Midway where it becomes a hazard to the bird population of the island. Midway Atoll is home to two-thirds (1.5 million) of the global population of Laysan Albatross. Nearly all of these albatross have plastic in their digestive system and one-third of their chicks die. Toxic additives used in the manufacture of plastic materials can leach out into their surroundings when exposed to water. Waterborne hydrophobic pollutants collect and magnify on the surface of plastic debris, thus making plastic far more deadly in the ocean than it would be on land. Hydrophobic contaminants are also known to bioaccumulate in fatty tissues, biomagnifying up the food chain and putting pressure on apex predators. Some plastic additives are known to disrupt the endocrine system when consumed; others can suppress the immune system or decrease reproductive rates. Floating debris can also absorb persistent organic pollutants from seawater, including PCBs, DDT and PAHs.

Toxins: Apart from plastics, there are particular problems with other toxins that do not disintegrate rapidly in the marine environment. Examples of persistent toxins are PCBs, DDT, pesticides, furans, dioxins, phenols and radioactive waste. Heavy metals are metallic chemical elements that have a relatively high density and are toxic or poisonous at low concentrations. Examples are mercury, lead, nickel, arsenic and cadmium. Such toxins can accumulate in the tissues of many species of aquatic life in a process called bioaccumulation. They are also known to accumulate in benthic environments, such as estuaries and bay muds: a geological record of human activities of the last century.

Specific examples

- Chinese and Russian industrial pollution such as phenols and heavy metals in the Amur River have devastated fish stocks and damaged its estuary soil.
- Wabamun Lake in Alberta, Canada, once the best whitefish lake in the area, now has unacceptable levels of heavy metals in its sediment and fish.
- Acute and chronic pollution events have been shown to impact southern California kelp forests, though the intensity of the impact seems to depend on both the nature of the contaminants and duration of exposure.
- Due to their high position in the food chain and the subsequent accumulation of heavy metals from their diet, mercury levels can be high in larger species such as bluefin and

albacore. As a result, in March 2004 the United States FDA issued guidelines recommending that pregnant women, nursing mothers and children limit their intake of tuna and other types of predatory fish.

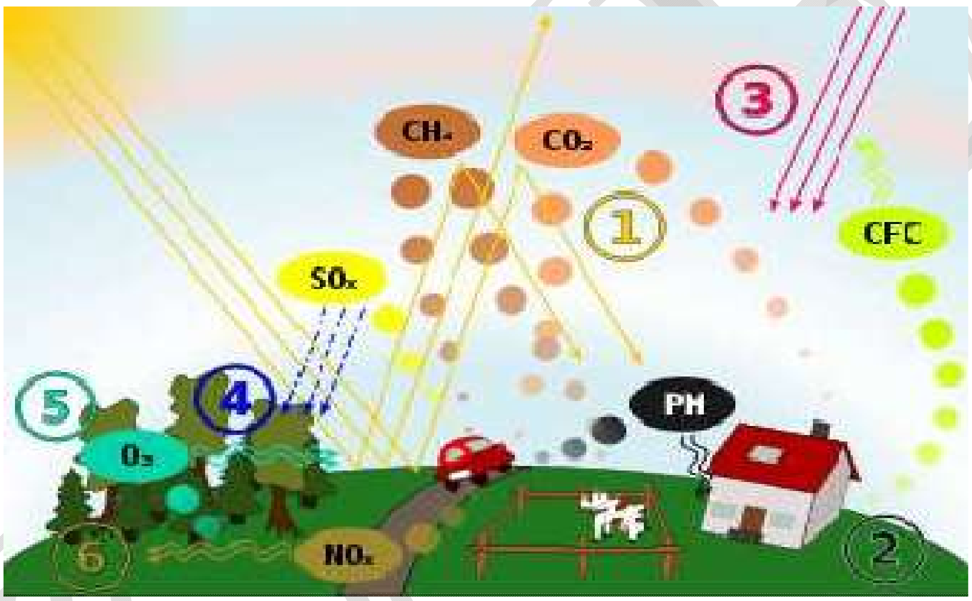
- Some shellfish and crabs can survive polluted environments, accumulating heavy metals or toxins in their tissues. For example, mitten crabs have a remarkable ability to survive in highly modified aquatic habitats, including polluted waters. The farming and harvesting of such species needs careful management if they are to be used as a food.
- Surface runoff of pesticides can alter the gender of fish species genetically, transforming male into female fish.
- Heavy metals enter the environment through oil spills - such as the Prestige oil spill on the Galician coast - or from other natural or anthropogenic sources.
- In 2005, the 'Ndrangheta, an Italian mafia syndicate, was accused of sinking at least 30 ships loaded with toxic waste, much of it radioactive. This has led to widespread investigations into radioactive-waste disposal rackets.
- Since the end of World War II, various nations, including the Soviet Union, the United Kingdom, the United States, and Germany, have disposed of chemical weapons in the Baltic Sea, raising concerns of environmental contamination.

Underwater noise: Marine life can be susceptible to noise or sound pollution from sources such as passing ships, oil exploration seismic surveys, and naval low-frequency active sonar. Sound travels more rapidly and over larger distances in the sea than in the atmosphere. Marine animals, such as cetaceans, often have weak eyesight, and live in a world largely defined by acoustic information. This applies also to many deeper sea fish, who live in a world of darkness. Between 1950 and 1975, ambient noise in the ocean increased by about ten decibels (that is a tenfold increase). Noise also makes species communicate louder, which is called the Lombard vocal response. Whale songs are longer when submarine-detectors are on. If creatures don't "speak" loud enough, their voice can be masked by anthropogenic sounds. These unheard voices might be warnings, finding of prey, or preparations of net-bubbling. When one species begins speaking louder, it will mask other species voices, causing the whole ecosystem to eventually speak louder. According to the oceanographer Sylvia Earle, "Undersea noise pollution is like the death of a thousand cuts. Each sound in itself may not be a matter of critical concern, but taken all together, the noise from shipping, seismic surveys, and military activity is creating a totally different environment than existed even 50 years ago. That high level of noise is bound to have a hard, sweeping impact on life in the sea.

Adaptation and mitigation: Much anthropogenic pollution ends up in the ocean. The 2011 edition of the United Nations Environment Programme Year Book identifies as the main emerging environmental issues the loss to the oceans of massive amounts of phosphorus, "a valuable fertilizer needed to feed a growing global population", and the impact billions of pieces of plastic waste are having globally on the health of marine environments. Bjorn Jennssen (2003) notes in his article, –Anthropogenic pollution may reduce biodiversity and productivity of marine ecosystems, resulting in reduction and depletion of human marine food resources‖. There are two ways the overall level of this pollution can be mitigated: either the human population is reduced, or a way is found to reduce the ecological footprint left behind by the average human. If the second way is not adopted, then the first way may be imposed as world ecosystems falter. The second way is for humans, individually, to pollute less. That requires social and political will, together with a shift in awareness so more people respect the environment and are less disposed to abuse it. At an operational level, regulations, and international government participation is needed. It is often very difficult to regulate marine pollution because pollution spreads over international barriers, thus making regulations hard to create as well as enforce.

Without appropriate awareness of marine pollution, the necessary global will to effectively address the issues may prove inadequate. Balanced information on the sources and harmful effects of marine pollution need to become part of general public awareness, and ongoing research is required to fully establish, and keep current, the scope of the issues. As expressed in Daoji and Dag’s research, one of the reasons why environmental concern is lacking among the Chinese is because the public awareness is low and therefore should be targeted. Likewise, regulation, based upon such in-depth research should be employed. In California, such regulations have already been put in place to protect Californian coastal waters from agricultural runoff. This includes the California Water Code, as well as several voluntary programs. Similarly, in India, several tactics have been employed that help reduce marine pollution, however, they do not significantly target the problem. In Chennai, sewage has been dumped further into open waters. Due to the mass of waste being deposited, open-ocean is best for diluting, and dispersing pollutants, thus making them less harmful to marine ecosystems.

Air pollution: Air pollution is the introduction into the atmosphere of chemicals, particulates, or biological materials that cause discomfort, disease, or death to humans, damage other living organisms such as food crops, or damage the natural environment or built environment. The atmosphere is a complex dynamic natural gaseous system that is essential to support life on planet Earth. Stratospheric ozone depletion due to air pollution has long been recognized as a threat to human health as well as to the Earth's ecosystems. Indoor air pollution and urban air quality are listed as two of the World’s Worst Toxic Pollution Problems in the 2008 Blacksmith Institute World's Worst Polluted Places report. A substance in the air that can be adverse to humans and the environment is known as an air pollutant. Pollutants can be in the form of solid particles, liquid droplets, or gases. In addition, they may be natural or man-made. Pollutants can be classified as primary or secondary. Usually, primary pollutants are directly produced from a process, such as ash from a volcanic eruption, the carbon monoxide gas from a motor vehicle exhaust or sulfur dioxide released from factories. Secondary pollutants are not emitted directly. Rather, they form in the air when primary pollutants react or interact. An important example of a secondary pollutant is ground level ozone one of the many secondary pollutants that make up photochemical smog. Some pollutants may be both primary and secondary: that is, they are both emitted directly and formed from other primary pollutants.



Schematic drawing, causes and effects of air pollution: (1) greenhouse effect, (2) particulate contamination, (3) increased UV radiation, (4) acid rain, (5) increased ground level ozone concentration, (6) increased levels of nitrogen oxides. Major primary pollutants produced by human activity include:

- Sulfur oxides (SO_x) - especially sulfur dioxide, a chemical compound with the formula SO_2. SO_2 is produced by volcanoes and in various industrial processes. Since coal and petroleum often contain sulfur compounds, their combustion generates sulfur dioxide. Further oxidation of SO_2, usually in the presence of a catalyst such as NO_2, forms H_2SO_4, and thus acid rain. This is one of the causes for concern over the environmental impact of the use of these fuels as power sources.
- Nitrogen oxides (NO_x) - especially nitrogen dioxide are expelled from high temperature combustion, and are also produced naturally during thunderstorms by electric discharge.

Can be seen as the brown haze dome above or plume downwind of cities. Nitrogen dioxide is the chemical compound with the formula NO_2. It is one of the several nitrogen oxides. This reddish-brown toxic gas has a characteristic sharp, biting odor. NO_2 is one of the most prominent air pollutants.

- Carbon monoxide (CO) - is a colourless, odourless, non-irritating but very poisonous gas. It is a product by incomplete combustion of fuel such as natural gas, coal or wood. Vehicular exhaust is a major source of carbon monoxide.
- Volatile organic compounds - VOCs are an important outdoor air pollutant. In this field they are often divided into the separate categories of methane (CH_4) and non-methane (NMVOCs). Methane is an extremely efficient greenhouse gas which contributes to enhanced global warming. Other hydrocarbon VOCs are also significant greenhouse gases via their role in creating ozone and in prolonging the life of methane in the atmosphere, although the effect varies depending on local air quality. Within the NMVOCs, the aromatic compounds benzene, toluene and xylene are suspected carcinogens and may lead to leukemia through prolonged exposure. 1, 3-butadiene is another dangerous compound which is often associated with industrial uses.
- Particulates, alternatively referred to as particulate matter (PM), atmospheric particulate matter, or fine particles, are tiny particles of solid or liquid suspended in a gas. In contrast, aerosol refers to particles and the gas together. Sources of particulates can be manmade or natural. Some particulates occur naturally, originating from volcanoes, dust storms, forest and grassland fires, living vegetation, and sea spray. Human activities, such as the burning of fossil fuels in vehicles, power plants and various industrial processes also generate significant amounts of aerosols. Averaged over the globe, anthropogenic aerosols—those made by human activities—currently account for about 10 percent of the total amount of aerosols in our atmosphere. Increased levels of fine particles in the air are linked to health hazards such as heart disease altered lung function and lung cancer.
- Persistent free radicals connected to airborne fine particles could cause cardiopulmonary disease.
- Toxic metals, such as lead and mercury, especially their compounds.
- Chlorofluorocarbons (CFCs) - harmful to the ozone layer emitted from products currently banned from use.
- Ammonia (NH_3) - emitted from agricultural processes. Ammonia is a compound with the formula NH_3. It is normally encountered as a gas with a characteristic pungent odor. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by

serving as a precursor to foodstuffs and fertilizers. Ammonia, either directly or indirectly, is also a building block for the synthesis of many pharmaceuticals. Although in wide use, ammonia is both caustic and hazardous.

- Odors — such as from garbage, sewage, and industrial processes
- Radioactive pollutants - produced by nuclear explosions, nuclear events, war explosives, and natural processes such as the radioactive decay of radon.
- Particulates created from gaseous primary pollutants and compounds in photochemical smog. Smog is a kind of air pollution; the word "smog" is a portmanteau of smoke and fog. Classic smog results from large amounts of coal burning in an area caused by a mixture of smoke and sulfur dioxide. Modern smog does not usually come from coal but from vehicular and industrial emissions that are acted on in the atmosphere by ultraviolet light from the sun to form secondary pollutants that also combine with the primary emissions to form photochemical smog.
- Ground level ozone (O_3) formed from NO_x and VOCs. Ozone (O_3) is a key constituent of the troposphere. It is also an important constituent of certain regions of the stratosphere commonly known as the Ozone layer. Photochemical and chemical reactions involving it drive many of the chemical processes that occur in the atmosphere by day and by night. At abnormally high concentrations brought about by human activities (largely the combustion of fossil fuel), it is a pollutant, and a constituent of smog.
- Peroxyacetyl nitrate (PAN) - similarly formed from NO_x and VOCs.

Minor air pollutants include:

- A large number of minor hazardous air pollutants. Some of these are regulated in USA under the Clean Air Act and in Europe under the Air Framework Directive
- A variety of persistent organic pollutants, which can attach to particulates

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Because of this, they have been observed to persist in the environment, to be capable of long-range transport, bioaccumulate in human and animal tissue, biomagnify in food chains, and to have potential significant impacts on human health and the environment.

Sources

Sources of air pollution refers to the various locations, activities or factors which are responsible for the releasing of pollutants into the atmosphere. These sources can be classified into two major categories which are:

Anthropogenic sources (man-made sources) mostly related to burning different kinds of fuel

- "Stationary Sources" include smoke stacks of power plants, manufacturing facilities (factories) and waste incinerators, as well as furnaces and other types of fuel-burning heating devices. In developing and poor countries, traditional biomass burning is the major source of air pollutants; traditional biomass includes wood, crop waste and dung.
- "Mobile Sources" include motor vehicles, marine vessels, aircraft and the effect of sound etc.
- Chemicals, dust and controlled burn practices in agriculture and forest management. Controlled or prescribed burning is a technique sometimes used in forest management, farming, prairie restoration or greenhouse gas abatement. Fire is a natural part of both forest and grassland ecology and controlled fire can be a tool for foresters. Controlled burning stimulates the germination of some desirable forest trees, thus renewing the forest.
- Fumes from paint, hair spray, varnish, aerosol sprays and other solvents.
- Waste deposition in landfills, which generate methane. Methane is highly flammable and may form explosive mixtures with air. Methane is also anasphyxiant and may displace oxygen in an enclosed space. Asphyxia or suffocation may result if the oxygen concentration is reduced to below 19.5% by displacement.
- Military, such as nuclear weapons, toxic gases, germ warfare and rocketry

Natural sources

- Dust from natural sources, usually large areas of land with few or no vegetation
- Methane, emitted by the digestion of food by animals, for example cattle
- Radon gas from radioactive decay within the Earth's crust. Radon is a colorless, odorless, naturally occurring, radioactive noble gas that is formed from the decay of radium. It is considered to be a health hazard. Radon gas from natural sources can accumulate in buildings, especially in confined areas such as the basement and it is the second most frequent cause of lung cancer, after cigarette smoking.
- Smoke and carbon monoxide from wildfires.

- Vegetation, in some regions, emits environmentally significant amounts of VOCs on warmer days. These VOCs react with primary anthropogenic pollutants—specifically, NO_x, SO_2, and anthropogenic organic carbon compounds—to produce a seasonal haze of secondary pollutants.
- Volcanic activity, which produce sulfur, chlorine, and ash particulates.

Emission factors: Air pollutant emission factors are representative values that people attempt to relate the quantity of a pollutant released to the ambient air with an activity associated with the release of that pollutant. These factors are usually expressed as the weight of pollutant divided by a unit weight, volume, distance, or duration of the activity emitting the pollutant (e.g., kilograms of particulate emitted per tonne of coal burned). Such factors facilitate estimation of emissions from various sources of air pollution. In most cases, these factors are simply averages of all available data of acceptable quality, and are generally assumed to be representative of long-term averages. There are 12 compounds in the list of POPs. Dioxins and furans are two of them and are intentionally created by combustion of organics, like open burning of plastics. The POPs are also endocrine disruptor and can mutate the human genes.

Indoor air quality (IAQ) : A lack of ventilation indoors concentrates air pollution where people often spend the majority of their time. Radon (Rn) gas, a carcinogen, is exuded from the Earth in certain locations and trapped inside houses. Building materials including carpeting and plywood emit formaldehyde (H_2CO) gas. Paint and solvents give off volatile organic compounds (VOCs) as they dry. Lead paint can degenerate into dust and be inhaled. Intentional air pollution is introduced with the use of air fresheners, incense, and other scented items. Controlled wood fires in stoves and fireplaces can add significant amounts of smoke particulates into the air, inside and out. Indoor pollution fatalities may be caused by using pesticides and other chemical sprays indoors without proper ventilation. Carbon monoxide (CO) poisoning and fatalities are often caused by faulty vents and chimneys, or by the burning of charcoal indoors. Chronic carbon monoxide poisoning can result even from poorly adjusted pilot lights. Traps are built into all domestic plumbing to keep sewer gas and hydrogen sulfide, out of interiors. Clothing emits tetrachloroethylene, or other dry cleaning fluids, for days after dry cleaning. Though its use has now been banned in many countries, the extensive use of asbestos in industrial and domestic environments in the past has left a potentially very dangerous material in many localities. Asbestosis is a chronic inflammatory medical condition affecting the tissue of the lungs. It occurs after long-term, heavy exposure to asbestos from asbestos-containing materials in structures. Sufferers have severe dyspnea (shortness of breath) and are at an increased risk regarding several different types of lung cancer. As clear explanations are not always stressed in non-technical literature, care should be taken to distinguish between several forms of relevant diseases. According to the World Health Organisation (WHO), these may defined as; asbestosis, *lung cancer*, and *Peritoneal Mesothelioma* (generally a very rare form of cancer, when more widespread it is almost always associated with prolonged exposure to asbestos). Biological sources of air pollution are also found indoors, as gases and airborne particulates. Pets produce dander, people produce dust from minute skin flakes and decomposed hair, dust mites in bedding, carpeting and furniture produce enzymes and micrometre-sized fecal droppings, inhabitants emit methane, mold forms in walls and generates mycotoxins and spores, air conditioning systems can incubate Legionnaires' disease and mold, and houseplants, soil and surrounding gardens can produce pollen, dust, and mold. Indoors, the lack of air circulation allows these airborne pollutants to accumulate more than they would otherwise occur in nature.

Health effects: Air pollution is a significant risk factor for multiple health conditions including respiratory infections, heart disease, and lung cancer, according to the WHO. The health effects caused by air pollution may include difficulty in breathing, wheezing, coughing, asthma and aggravation of existing respiratory and cardiac conditions. These effects can result in increased medication use, increased doctor or emergency room visits, more hospital admissions and premature death. The human health effects of poor air quality are far reaching, but principally affect the body's respiratory system and the cardiovascular system. Individual reactions to air pollutants depend on the type of pollutant a person is exposed to, the degree of exposure, the individual's health status and genetics. The most common sources of air pollution include particulates, ozone, nitrogen dioxide, and sulfur dioxide. Both indoor and outdoor air pollution have caused approximately 3.3 million deaths worldwide. Children aged less than five years that live in developing countries are the most vulnerable population in terms of total deaths attributable to indoor and outdoor air pollution. The World Health Organization states that 2.4 million people die each year from causes directly attributable to air pollution, with 1.5 million of these deaths attributable to indoor air pollution. "Epidemiological studies suggest that more than 500,000 Americans die each year from cardiopulmonary disease linked to breathing fine particle air pollution. A study by the University of Birmingham has shown a strong correlation between pneumonia related deaths and air pollution from motor vehicles. Worldwide more deaths per year are linked to air pollution than to automobile accidents. A 2005 study by the European Commission calculated that air pollution reduces life expectancy by an average of almost nine months across the European Union. [Causes of deaths include aggravated asthma, emphysema, lung and heart diseases, and respiratory allergies. The US EPA estimates that a proposed set of changes in diesel engine technology (*Tier 2*) could result in 12,000 fewer *premature mortalities*, 15,000 fewer heart attacks, 6,000 fewer emergency room visits by children with asthma, and 8,900 fewer respiratory-related hospital admissions each year in the

Effects on cardiovascular health: A 2007 review of evidence found ambient air pollution exposure is a risk factor correlating with increased total mortality from cardiovascular events (range: 12% to 14% per a 10 microg/m^3 increase). Air pollution is also emerging as a risk factor for stroke, particularly in developing countries where pollutant levels are highest. A 2007 study found that in women air pollution is associated not with hemorrhagic but with ischemic stroke. Air pollution was also found to be associated with increased incidence and mortality from coronary stroke in a cohort study in 2011. Associations are believed to be causal and effects may be mediated by vasoconstriction, low-grade inflammation or autonomic nervous system imbalance or other mechanisms.

Effects on cystic fibrosis: A study from around the years of 1999 to 2000, by the University of Washington, showed that patients near and around particulates air pollution had an increased risk of pulmonary exacerbations and decrease in lung function. Patients were examined before the study for amounts of specific pollutants like *Pseudomonas aeruginosa* or *Burkholderia cenocepacia* as well as their socioeconomic standing. Participants involved in the study were located in the United States in close proximity to an Environmental Protection Agency.[*clarification needed*] During the time of the study 117 deaths were associated with air pollution. Many patients in the study lived in or near large metropolitan areas in order to be close to medical help. These same patients had higher level of pollutants found in their system because of more emissions in larger cities. As cystic fibrosis patients already suffer from decreased lung function, everyday pollutants such as smoke, emissions from automobiles, tobacco smoke and improper use of indoor heating devices could further compromise lung function.

Effects on COPD and asthma: Chronic obstructive pulmonary disease (COPD) includes diseases such as chronic bronchitis and emphysema. Researchers have demonstrated increased risk of developing asthma and COPD from increased exposure to traffic-related air pollution. Additionally, air pollution has been associated with increased hospitalizations and mortality from asthma and COPD. A study conducted in 1960-1961 in the wake of the Great Smog of 1952 compared 293 London residents with 477 residents of Gloucester, Peterborough, and Norwich, three towns with low reported death rates from chronic bronchitis. All subjects were male postal truck drivers aged 40 to 59. Compared to the subjects from the outlying towns, the London subjects exhibited more severe respiratory symptoms (including cough, phlegm, and dyspnea), reduced lung function (FEV_1 and peak flow rate), and increased sputum production and purulence. The differences were more pronounced for subjects aged 50 to 59. The study controlled for age and smoking habits, so concluded that air pollution was the most likely cause of the observed differences. It is believed that much like cystic fibrosis, by living in a more urban environment serious health hazards become more apparent. Studies have shown that in urban areas patients suffer mucus hypersecretion, lower levels of lung function, and more self diagnosis of chronic bronchitis and emphysema.

Control devices

The following items are commonly used as pollution control devices by industry or transportation devices. They can either destroy contaminants or remove them from an exhaust stream before it is emitted into the atmosphere.

Particulate control

1. Mechanical collectors (dust cyclones, multicyclones)
2. Electrostatic precipitators- An electrostatic precipitator (ESP), or electrostatic air cleaner is a particulate collection device that removes particles from a flowing gas (such as air) using the force of an induced electrostatic charge. Electrostatic precipitators are highly efficient filtration devices that minimally impede the flow of gases through the device, and can easily remove fine particulates such as dust and smoke from the air stream.
3. Baghouses - Designed to handle heavy dust loads, a dust collector consists of a blower, dust filter, a filter-cleaning system, and a dust receptacle or dust removal system (distinguished from air cleaners which utilize disposable filters to remove the dust). Particulate scrubbers Wet scrubber is a form of pollution control technology. The term describes a variety of devices that use pollutants from a furnace flue gas or from other gas streams. In a wet scrubber, the polluted gas stream is brought into contact with the scrubbing liquid, by spraying it with the liquid, by forcing it through a pool of liquid, or by some other contact method, so as to remove the pollutants.

- **Scrubbers**
    - Baffle spray scrubber
    - Cyclonic spray scrubber
    - Ejector venturi scrubber
    - Mechanically aided scrubber
    - Spray tower
    - Wet scrubber
- **NOx control**
    - Low NOx burners
    - Selective catalytic reduction (SCR)

- Selective non-catalytic reduction (SNCR)
- NOx scrubbers
- Exhaust gas recirculation
- Catalytic converter (also for VOC control)

- **VOC abatement**
    - Adsorption systems, such as activated carbon
    - Flares
    - Thermal oxidizers
    - Catalytic converters
    - Biofilters
    - Absorption (scrubbing)
    - Cryogenic condensers
- **Acid Gas/SO_2 control**
    - Wet scrubbers
    - Dry scrubbers
    - Flue-gas desulfurization
- **Mercury control**
    - Sorbent Injection Technology
    - Electro-Catalytic Oxidation (ECO)
    - K-Fuel

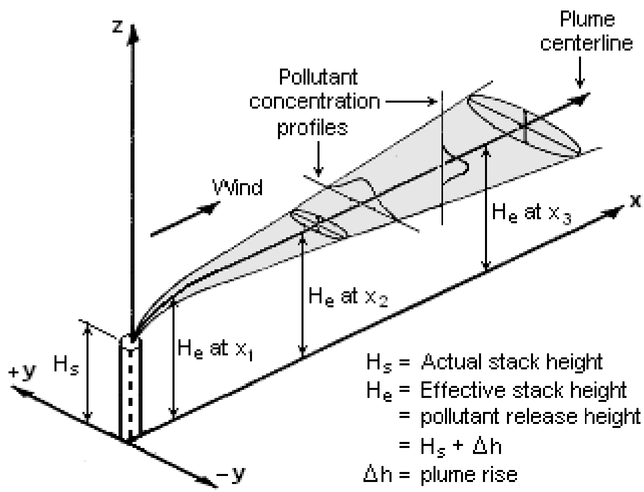
Atmospheric dispersion

The basic technology for analyzing air pollution is through the use of a variety of mathematical models for predicting the transport of air pollutants in the lower atmosphere.

The principal methodologies are

Point source dispersion, used for industrial sources.

- Line source dispersion, used for airport and roadway air dispersion modeling
- Area source dispersion, used for forest fires or dust storms
- Photochemical models, used to analyze reactive pollutants that form smog
- The point source problem is the best understood, since it involves simpler mathematics and has been studied for a long period of time, dating back to about the year 1900. It uses a Gaussian dispersion model for continuous buoyant pollution plumes to predict the air pollution isopleths, with consideration given to wind velocity, stack height, emission rate and stability class (a measure of atmospheric turbulence). This model has been extensively validated and calibrated with experimental data for all sorts of atmospheric conditions.



Visualization of a buoyant Gaussian air pollution dispersion plume as used in many atmospheric dispersion models.

The roadway air dispersion model was developed starting in the late 1950s and early 1960s in response to requirements of the National Environmental Policy Act and the U.S. Department of Transportation (then known as the Federal Highway Administration) to understand impacts of proposed new highways upon air quality, especially in urban areas. Several research groups were active in this model development, among which were: the Environmental Research and Technology (ERT) group in Lexington, Massachusetts, the ESL Inc. group in Sunnyvale, California and the California Air Resources Board group in Sacramento, California. The research of the ESL group received a boost with a contract award from the United States Environmental Protection Agency to validate a line source model using sulfur hexafluoride as a tracer gas. This program was successful in validating the line source model developed by ESL Inc. Some of the earliest uses of the model were in court cases involving highway air pollution, the Arlington, Virginia portion of Interstate 66 and the New Jersey Turnpike widening project through East Brunswick, New Jersey. Area source models were developed in 1971 through 1974 by the ERT and ESL groups, but addressed a smaller fraction of total air pollution emissions, so that their use and need was not as widespread as the line source model, which enjoyed hundreds of different applications as early as the 1970s.

Microbial biodegradation

Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to clean up contaminated environments. Thesebioremediation and biotransformation methods endeavour to harness the astonishing, naturally occurring ability of microbial xenobiotic metabolism to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), heterocyclic compounds (such as pyridine or quinoline), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of organisms to adapt to changing environmental conditions. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to degrade or convert such compounds. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information. In the field of Environmental microbiology,genome-based global studies open a new era providing unprecedented *in silico* views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation strategies to changing environmental conditions. Functional genomic and metagenomic approaches are increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

Aerobic biodegradation of pollutants: The burgeoning amount of bacterial genomic data provides unparalleled opportunities for understanding the genetic and molecular bases of the degradation of organic pollutants. Aromatic compounds are among the most recalcitrant of these pollutants and lessons can be learned from the recent genomic studies of *Burkholderia xenovorans* LB400 and *Rhodococcus* sp. strain RHA1, two of the largest bacterial genomes completely sequenced to date. These studies have helped expand our understanding of bacterial catabolism, non-catabolic physiological adaptation toorganic compounds, and the evolution of large bacterial genomes. First, the metabolic pathways from phylogenetically diverse isolates are very similar with respect to overall organization. Thus, as originally noted in pseudomonads, a large number of "peripheral aromatic" pathways funnel a range of natural and xenobiotic compounds into a restricted number of "central aromatic" pathways. Nevertheless, these pathways are genetically organized in genus-specific fashions, as exemplified by the b-ketoadipate and Paa pathways. Comparative genomic studies further reveal that some pathways are more widespread than initially thought. Thus, the Box and Paa pathways illustrate the prevalence of non-oxygenolytic ring-cleavage strategies in aerobic aromatic degradation processes. Functional genomic studies have been useful in establishing that even organisms harboring high numbers of homologous enzymes seem to contain few examples of true redundancy. For example, the multiplicity of ring-cleaving dioxygenases in certain rhodococcal isolates may be attributed to the cryptic aromatic catabolism of different terpenoids and steroids. Finally, analyses have indicated that recent genetic flux appears to have played a more significant role in the evolution of some large genomes, such as LB400's, than others. However, the emerging trend is that the large gene repertoires of potent pollutant degraders such as LB400 and RHA1 have evolved principally through more ancient processes. That this is true in such phylogenetically diverse species is remarkable and further suggests the ancient origin of this catabolic capacity.

Anaerobic biodegradation of pollutants: Anaerobic microbial mineralization of recalcitrant organic pollutants is of great environmental significance and involves intriguing novel biochemical reactions. In particular, hydrocarbons and halogenated compounds have long been doubted to be degradable in the absence of oxygen, but the isolation of hitherto unknown anaerobic hydrocarbon-degrading and reductively dehalogenatingbacteria during the last decades provided ultimate proof for these processes in nature. While such research involved mostly chlorinated compounds initially, recent studies have revealed reductive dehalogenation of bromine and iodine moieties in aromatic pesticides.[4] Other reactions, such as biologically induced abiotic reduction by soil minerals, has been shown to deactivate relatively persistent aniline-based herbicides far more rapidly than observed in aerobic environments. Many novel biochemical reactions were discovered enabling the respective metabolic pathways, but progress in the molecular understanding of these bacteria was rather slow, since genetic systems are not readily applicable for most of them. However, with the increasing application of genomicsin the field of environmental microbiology, a new and promising perspective is now at hand to obtain molecular insights into these new metabolic properties. Several complete genome sequences were determined during the last few years from bacteria capable of anaerobic organic pollutant degradation. The ~4.7 Mb genome of the facultative denitrifying *Aromatoleum aromaticum* strain EbN1 was the first to be determined for an anaerobic hydrocarbon degrader (using toluene or ethylbenzene as substrates). The genome sequence revealed about two dozen gene clusters(including several paralogs) coding for a complex catabolic network for anaerobic and aerobic degradation of aromatic compounds. The genome sequence forms the basis for current detailed studies on regulation of pathways and enzyme structures. Further genomes of anaerobic hydrocarbon degrading bacteria were recently completed for the iron-reducing species *Geobacter metallireducens* (accession nr. NC_007517) and the perchlorate-reducing *Dechloromonas aromatica* (accession nr. NC_007298), but these are not yet evaluated in formal publications. Complete genomes were also determined for bacteria capable of anaerobic degradation of halogenated hydrocarbons by halorespiration: the ~1.4 Mb genomes of *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain CBDB1 and the ~5.7 Mb genome of *Desulfitobacterium hafniense* strain Y51. Characteristic for all these bacteria is the presence of multiple paralogous genes for reductive dehalogenases, implicating a wider dehalogenating spectrum of the organisms than previously known. Moreover, genome sequences provided unprecedented insights into the evolution of reductive dehalogenation and differing strategies for niche adaptation. Recently, it has become apparent that some organisms, including *Desulfitobacterium chlororespirans*, originally evaluated for halorespiration on chlorophenols, can also use certain brominated compounds, such as the herbicide bromoxynil and its major metabolite as electron acceptors for growth. Iodinated compounds may be dehalogenated as well, though the process may not satisfy the need for an electron acceptor

Bioavailability, chemotaxis, and transport of pollutants: Bioavailability, or the amount of a substance that is physiochemically accessible to microorganisms is a key factor in the efficient biodegradation of pollutants. O'Loughlin *et al.* (2000) showed that, with the exception of kaolinite clay, most soil clays and cation exchange resins attenuated biodegradation of 2-picoline by *Arthrobacter* sp. strain R1, as a result of adsorption of the substrate to the clays. Chemotaxis, or the directed movement of motile organisms towards or away from chemicals in the environment is an important physiological response that may contribute to effective catabolism of molecules in the environment. In addition, mechanisms for the intracellular accumulation of aromatic molecules via various transport mechanisms are also important.

Oil biodegradation: Petroleum oil contains aromatic compounds that are toxic for most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, about 250 million liters of petroleum enter the marine environment every year from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB). *Alcanivorax borkumensis* was the first HCB to have its genome sequenced. In addition to hydrocarbons, crude oil often contains various heterocyclic compounds, such as pyridine, which appear to be degraded by similar, though separate mechanisms than hydrocarbons.

Cholesterol biodegradation: Many synthetic steroidic compounds like some sexual hormones frequently appear in municipal and industrial wastewaters, acting as environmental pollutants with strong metabolic activities negatively affecting the ecosystems. Since these compounds are common carbon sources for many different microorganisms their aerobic and anaerobic mineralization has been extensively studied. The interest of these studies lies on the biotechnological applications of sterol transforming enzymes for the industrial synthesis of sexual hormones and corticoids. Very recently the catabolism of cholesterol has acquired a high relevance because it is involved in the infectivity of *Mycobacterium tuberculosis*.

Analysis of waste biotreatment: Sustainable development requires the promotion of environmental management and a constant search for new technologies to treat vast quantities of wastes generated by increasinganthropogenic activities. Biotreatment, the processing of wastes using living organisms, is an environmentally friendly, relatively simple and cost-effective alternative to physico-chemical clean-up options. Confined environments, such as bioreactors, have been engineered to overcome the physical, chemical and biological limiting factors of biotreatment processes in highly controlled systems. The great versatility in the design of confined environments allows the treatment of a wide range of wastes under optimized conditions. To perform a correct assessment, it is necessary to consider various microorganisms having a variety of genomes and expressed transcripts and proteins. A great number of analyses are often required. Using traditional genomic techniques, such assessments are limited and time-consuming. However, several high-throughput techniques originally developed for medical studies can be applied to assess biotreatment in confined environments.

Metabolic engineering and biocatalytic applications: The study of the fate of persistent organic chemicals in the environment has revealed a large reservoir of enzymatic reactions with a large potential in preparative organic synthesis, which has already been exploited for a number of oxygenases on pilot and even on industrial scale. Novel catalysts can be obtained from metagenomic libraries and DNA sequence based approaches. Our increasing capabilities in adapting the catalysts to specific reactions and process requirements by rational and random mutagenesis broadens the scope for application in the fine chemical industry, but also in the field of biodegradation. In many cases, these catalysts need to be exploited in whole cell bioconversions or in fermentations, calling for system-wide approaches to understanding strain physiology and metabolism and rational approaches to the engineering of whole cells as they are increasingly put forward in the area of systems biotechnology and synthetic biology.

Fungal biodegradation: In the ecosystem, different substrates are attacked at different rates by consortia of organisms from different kingdoms. *Aspergillus* and other moulds play an important role in these consortia because they are adept at recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some aspergilli are capable of degrading more refractory compounds such as fats, oils, chitin, and keratin. Maximum decomposition occurs when there is sufficient nitrogen, phosphorus and other essential inorganic nutrients. Fungi also provide food for many soil organisms. For *Aspergillus* the process of degradation is the means of obtaining nutrients. When these moulds degrade human-made substrates, the process usually is called biodeterioration. Both paper and textiles (cotton, jute, and linen) are particularly vulnerable to *Aspergillus* degradation. Our artistic heritage is also subject to *Aspergillus* assault. To give but one example, after Florence in Italy flooded in 1969, 74% of the isolates from a damaged Ghirlandaio fresco in the Ognissanti church were *Aspergillus versicolor*.

Bioleaching : Bioleaching is the extraction of metals from their ores through the use of living organisms. This is much cleaner than the traditional heap leaching using cyanide. Bioleaching is one of several applications with biohydro metallurgy and several methods are used as recover copper, zinc, lead, arsenic, antimony, nickel, molybdenum, gold, silver and cobalat.

Process: Bioleaching can involve numerous ferrous iron and sulfur oxidizing bacteria, including *Acidithiobacillus ferrooxidans* and *Acidithiobacillus* (formerly known as *Thiobacillus*). As a general principle, Fe^{3+} ions are used to oxidize the ore. This step is entirely independent of microbes. The role of the bacteria is the further oxidation of the ore, but also the regeneration of the chemical oxidant Fe^{3+} from Fe^{2+}. For example, bacteria catalyse the breakdown of the mineral pyrite (FeS_2) by oxidising the sulfur and metal (in this case ferrous iron, (Fe^{2+}) using oxygen. This yields solubleproducts that can be further purified and refined to yield the desired metal.

Pyrite leaching (FeS_2): In the first step, disulfide is spontaneously oxidized to thiosulfate by ferric ion (Fe^{3+}), which in turn is reduced to give ferrous ion (Fe^{2+}):

(1) $FeS_2 + 6\ Fe^{3+} + 3\ H_2O \longrightarrow 7\ Fe^{2+} + S_2O_3^{2-} + 6\ H^+$ spontaneous

The ferrous ion is then oxidized by bacteria using oxygen:

(2) $4\ Fe^{2+} + O_2 + 4\ H^+ \longrightarrow 4\ Fe^{3+} + 2\ H_2O$ (iron oxidizers)

Thiosulfate is also oxidized by bacteria to give sulfate:

(3) $S_2O_3^{2-} + 2\ O_2 + H_2O \longrightarrow 2\ SO_4^{2-} + 2\ H^+$ (sulfur oxidizers)

The ferric ion produced in reaction (2) oxidized more sulfide as in reaction (1), closing the cycle and given the net reaction:

(4) $2\ FeS_2 + 7\ O_2 + 2\ H_2O \longrightarrow 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+$

The net products of the reaction are soluble ferrous sulfate and sulfuric acid.

The microbial oxidation process occurs at the cell membrane of the bacteria. The electrons pass into the cells and are used in biochemical processes to produce energy for the bacteria while reducing oxygen to water. The critical reaction is the oxidation of sulfide by ferric iron. The main role of the bacterial step is the regeneration of this reactant. The process for copper is very similar, but the efficiency and kinetics depend on the copper mineralogy. The most efficient minerals are supergene minerals such as chalcocite, Cu_2S and covellite, CuS. The main copper mineral chalcopyrite ($CuFeS_2$) is not leached very efficiently, which is why the dominant copper-producing technology remains flotation, followed by smelting and refining. The leaching of $CuFeS_2$ follows the two stages of being dissolved and then further oxidised, with Cu^{2+} ions being left in solution.

Chalcopyrite leaching:

(1) $CuFeS_2 + 4\ Fe^{3+} \longrightarrow Cu^{2+} + 5\ Fe^{2+} + 2\ S_0$ spontaneous

(2) $4\ Fe^{2+} + O_2 + 4\ H^+ \longrightarrow 4\ Fe^{3+} + 2\ H_2O$ (iron oxidizers)

(3) $2\ S^0 + 3\ O_2 + 2\ H_2O \longrightarrow 2\ SO_4^{2-} + 4\ H^+$ (sulfur oxidizers)

net reaction:

(4) $CuFeS_2 + 4\ O_2 \longrightarrow Cu^{2+} + Fe^{2+} + 2\ SO_4^{2-}$

In general, sulfides are first oxidized to elemental sulfur, whereas disulfides are oxidized to give thiosulfate, and the processes above can be applied to other sulfidic ores. Bioleaching of non-sulfidic ores such as pitchblende also uses ferric iron as an oxidant (e.g., $UO_2 + 2\ Fe^{3+}$ ==> $UO_2^{2+} + 2\ Fe^{2+}$). In this case, the sole purpose of the bacterial step is the regeneration of Fe^{3+}. Sulfidic iron ores can be added to speed up the process and provide a source of iron. Bioleaching of non-sulfidic ores by layering of waste sulfides and elemental sulfur, colonized by*Acidithiobacillus* spp., has been accomplished, which provides a strategy for accelerated leaching of materials that do not contain sulfide minerals.

The dissolved copper (Cu^{2+}) ions are removed from the solution by ligand exchange solvent extraction, which leaves other ions in the solution. The copper is removed by bonding to a ligand, which is a large molecule consisting of a number of smaller groups, each possessing a lone electron pair. The ligand-copper complex is extracted from the solution using an organic solvent such askerosene:

$Cu^{2+}_{(aq)} + 2LH(organic) \rightarrow CuL_2(organic) + 2H^+_{(aq)}$

The ligand donates electrons to the copper, producing a complex - a central metal atom (copper) bonded to the ligand. Because this complex has no charge, it is no longer attracted to polar water molecules and dissolves in the kerosene, which is then easily separated from the solution. Because the initial reaction is reversible, it is determined by pH. Adding concentrated acid reverses the equation, and the copper ions go back into an aqueous solution.Then the copper is passed through an electro-winning process to increase its purity: An electric current is passed through the resulting solution of copper ions. Because copper ions have a 2+ charge, they are attracted to the negative cathodes and collect there.The copper can also be concentrated and separated by displacing the copper with Fe from scrap iron:

$Cu^{2+}_{(aq)} + Fe_{(s)} \rightarrow Cu_{(s)} + Fe^{2+}_{(aq)}$

The electrons lost by the iron are taken up by the copper. Copper is the oxidising agent (it accepts electrons), and iron is the reducing agent (it loses electrons).Traces of precious metals such as gold may be left in the original solution. Treating the mixture with sodium cyanide in the presence of free oxygen dissolves the gold. The gold is removed from the solution by adsorbing (taking it up on the surface) to charcoal.

Bioleaching with fungi

Several species of fungi can be used for bioleaching. Fungi can be grown on many different substrates, such as electronic scrap, catalytic converters, and fly ash from municipal wasteincineration. Experiments have shown that two fungal strains (*Aspergillus niger, Penicillium simplicissimum*) were able to mobilize Cu and Sn by 65%, and Al, Ni, Pb, and Zn by more than 95%.*Aspergillus niger* can produce some organic acids such as citric acid. This form of leaching does not rely on microbial oxidation of metal but rather uses microbial metabolism as source of acids that directly dissolve the metal.

Extractions involve many expensive steps such as roasting and smelting, which require sufficient concentrations of elements in ores and are environmentally unfriendly. Low concentrations are not a problem for bacteria because they simply ignore the waste that surrounds the metals, attaining extraction yields of over 90% in some cases. These microorganisms actually gain energy by breaking down minerals into their constituent elements. The company simply collects the ions out of the solution after the bacteria have finished. There is a limited amount of ores.

Advantages of bioleaching

Economical: bioleaching is in general simpler and, therefore, cheaper to operate and maintain than traditional processes, since fewer specialists are needed to operate complex chemical plants.

Environmental: The process is more environmentally friendly than traditional extraction methods. For the company this can translate into profit, since the necessary limiting of sulfur dioxide emissions during smelting is expensive. Less landscape damage occurs, since the bacteria involved grow naturally, and the mine and surrounding area can be left relatively untouched. As the bacteria breed in the conditions of the mine, they are easily cultivated and recycled.

Ore concentration: Bioleaching can extract metals from ores that are too poor for other technologies. It can be used to partially replace the extensive crushing and grinding that translates to prohibitive cost and energy consumption in a conventional process.

Disadvantages of bioleaching

Economical: The bacterial leaching process is very slow compared to smelting. This brings in less profit as well as introducing a significant delay in cash flow for new plants.

Environmental: Toxic chemicals are sometimes produced in the process. Sulfuric acid and H^+ ions that have been formed can leak into the ground and surface water turning it acidic, causing environmental damage. Heavy ions such as iron, zinc, and arsenic leak during acid mine drainage. When the pH of this solution rises, as a result of dilution by fresh water, these ions precipitate, forming "Yellow Boy" pollution. For these reasons, a setup of bioleaching must be carefully planned, since the process can lead to a biosafety failure. Unlike other methods, once started, bioheap leaching cannot be quickly stopped, because leaching would still continue with rainwater and natural bacteria. At the current time, it is more economical to smelt copper ore rather than to use bioleaching, since the concentration of copper in its ore is in general quite high. The profit obtained from the speed and yield of smelting justifies its cost. Nonetheless, at the largest copper mine of the world, Escondida in Chile the process seems to be favorable. However, the concentration of gold in its ore is in general very low. The lower cost of bacterial leaching in this case outweighs the time it takes to extract the metal. Economically it is also very expensive and many companies once started can not keep up with the demand and end up in debt. Projects like Finnish Talvivaara proved to be environmentally and economically disasters.

Oxidation of Minerals: The minerals in rocks formed beneath the surface are in equilibrium with the temperature and pressure conditions at time of their formation and thus are quite stable. However, many minerals are no longer in equilibrium with their environmental conditions when exposed at the surface and are susceptible to weathering. Chemical weathering results in the formation and retention of minerals in equilibrium with environmental conditions at the Earth's surface. The least stable minerals in igneous and metamorphic rocks are olivine and plagioclase, the most stable is quartz. The interlocking and spacing of mineral grains controls the tendency towards weathering. Rocks with loosely interlocking mineral grains allow agents of chemical weathering to penetrate, thus speeding their decomposition. Limestone is primarily composed of calcite, a mineral that is quite soluble under surface conditions and easily dissolves in humid environments. In dry regions, the tight texture of limestone prevents it from disintegration and thus is a relatively resistant rock when found in deserts.

The process of chemical weathering tends to:

- increase bulk creating stress within rocks
- lower the density minerals
- decreased particle size resulting in increased surface area
- creates more mobile materials

- creates more stable minerals
- Chemical weathering processes
- Oxidation takes place when oxygen reacts with earth materials. Oxygen dissolved in water combines with atoms of metallic elements abundant in silicate minerals. Attacking metals in the soil, oxidation causes them to rust leaving the soil a brownish red to red color. When oxygen combines with iron, the reddish iron oxide hematite (Fe2O3) is formed: 4Fe+3 + 3O2 -> 2Fe2O3

Hydrolysis is an exchange reaction involving minerals and water. Free hydrogen (H+) and hydroxide (OH)- ions in water are able to replace mineral ions and drive them into solution. As a result, the mineral's atomic structure is changed into a new form. It is a process whereby silicate minerals like potassium feldspar are weathered and a clay mineral is formed.

$2KAlSi3O_8 + 2H+ + 9\ H2O \rightarrow Al2Si2O_5(OH)_4 + 4H4SiO4 + 2\ K_2+$

Hydration involves the absorption of water like which occurs during the conversion of hematite to limonite:

$2Fe2O_3 + 3H20 \rightarrow 2Fe2O_3.\ 3H20$

Some geoscientists question whether hydration is a true chemical weathering process because the process is readily reversible and the new product is not chemically different from its precursor. Some would rather call hydration a physical weathering process. Carbonic acid action involves combination of carbon dioxide and water. Though present in pure water, carbon dioxide dissolved in water provides ions that produces free hydrogen. Carbon dioxide in the atmosphere combines with rain water to form carbonic acid (H2CO3):

$H2O + CO_2 \rightarrow H2CO_3$

Though weak, when carbonic acid is combined with a mineral like calcite (CaCO3)common to limestone, calcium and bicarbonate ions are released and carried off by groundwater.

$CaCO_3 + H2CO_3 \rightarrow Ca+2 + 2\ HCO-3$

Rock resistance to weathering: Rocks react differently to weathering due to the differences in mineral content and structure. Some minerals are unstable under surface conditions and are readily soluble. Others are stable and resist the agents of weathering. Some rock-forming minerals are physically soft, being easily crushed and split while harder minerals are less easily broken apart. The arrangement and size of mineral grains control weathering processes. Water has a difficult time penetrating intricately locked and closely spaced mineral grains to promote weathering. Larger, loosely cemented minerals disintegrate and decompose more readily. Minerals in the form of poorly joined sheets readily break apart. Granite is a coarse grained rock composed of quartz and feldspar. Both quartz and feldspar are hard minerals, but feldspar is less stable under surface conditions than quartz. The feldspar readily weathers to become clay in humid conditions. The feldspar will weather in dry climates as the somewhat porous granite allows moisture to penetrate. As the feldspar decomposes it weakens the bonds holding the rock together and it disintegrate.

Oxidation: Within the weathering environment chemical oxidation of a variety of metals occurs. The most commonly observed is the oxidation of Fe^{2+} (iron) and combination with oxygen and water to form Fe^{3+} hydroxides and oxides such as goethite, limonite, and hematite. This gives the affected rocks a reddish-brown coloration on the surface which crumbles easily and weakens the rock. This process is better known as 'rusting', though it is distinct from the rusting of metallic iron. Many other metallic ores and minerals oxidize and hydrate to produce colored deposits, such as chalcopyrites or $CuFeS_2$ oxidizing to copper hydroxideand iron oxides

Biogeochemical weathering: Chemical weathering changes the composition of rocks, often transforming them when water interacts with minerals to create various chemical reactions. Chemical weathering is a gradual and ongoing process as the mineralogy of the rock adjusts to the near surface environment. New or *secondary minerals* develop from the original minerals of the rock. In this the processes of oxidation and hydrolysis are most important. Chemical weathering is enhanced by such geological agents as the presence of water and oxygen, as well as by such biological agents as the acids produced by microbial and plant-root metabolism.The process of mountain block uplift is important in exposing new rock strata to the atmosphere and moisture, enabling important chemical weathering to occur; significant release occurs of Ca^{2+} and other ions into surface waters.

Dissolution and carbonation: Rainfall is acidic because atmospheric carbon dioxide dissolves in the rainwater producing weak carbonic acid. In unpolluted environments, the rainfall pH is around 5.6. Acid rainoccurs when gases such as sulfur dioxide and nitrogen oxides are present in the atmosphere. These oxides react in the rain water to produce stronger acids and can lower the pH to 4.5 or even 3.0. Sulfur dioxide, SO_2, comes from volcanic eruptions or from fossil fuels, can become sulfuric acid within rainwater, which can cause solution weathering to the rocks on which it falls. Some minerals, due to their natural solubility (e.g. evaporites), oxidation potential (iron-rich minerals, such as pyrite), or instability relative to surficial conditions (see Goldich dissolution series) will weather through dissolution naturally, even without acidic water. One of the most well-known solution weathering processes is carbonation, the process in which atmospheric carbon dioxide leads to solution weathering. Carbonation occurs on rocks which contain calcium carbonate, such as limestone and chalk. This takes place when rain combines with carbon dioxide or an organic acid to form a weak carbonic acid which reacts with calcium carbonate (the limestone) and forms calcium bicarbonate. This process speeds up with a decrease in temperature, not because low temperatures generally drive reactions faster, but because colder water holds more dissolved carbon dioxide gas. Carbonation is therefore a large feature of glacial weathering.

The reactions as follows:

$CO_2 + H_2O \rightarrow H_2CO_3$

carbon dioxide + water → carbonic acid

$H_2CO_3 + CaCO_3 \rightarrow Ca(HCO_3)_2$

carbonic acid + calcium carbonate → calcium bicarbonate

Carbonation on the surface of well-jointed limestone produces a dissected limestone pavement. This process is most effective along the joints, widening and deepening them.

Hydration : Mineral hydration is a form of chemical weathering that involves the rigid attachment of H+ and OH- ions to the atoms and molecules of a mineral. When rock minerals take up water, the increased volume creates physical stresses within the rock. For example iron oxides are converted to iron hydroxides and the hydration of anhydrite forms gypsum. A freshly broken rock shows differential chemical weathering (probably mostly oxidation) progressing inward.

Hydrolysis on silicates and carbonates

Hydrolysis is a chemical weathering process affecting silicate and carbonate minerals. In such reactions, pure water ionizes slightly and reacts with silicate minerals. An example reaction:

$Mg_2SiO_4 + 4\ H^+ + 4\ OH^- \square\ 2\ Mg^{2+} + 4\ OH^- + H_4SiO_4$

olivine (forsterite) + four ionized water molecules □ ions in solution + silicic acid in solution

This reaction theoretically results in complete dissolution of the original mineral, if enough water is available to drive the reaction. In reality, pure water rarely acts as a H^+ donor. Carbon dioxide, though, dissolves readily in water forming a weak acid and H^+ donor.

$Mg_2SiO_4 + 4\ CO_2 + 4\ H_2O$ $2\ Mg^{2+} + 4\ HCO_3^- + H_4SiO_4$

olivine (forsterite) + carbon dioxide + water Magnesium and bicarbonate ions in solution + silicic acid in solution. This hydrolysis reaction is much more common. Carbonic acid is consumed by silicate weathering, resulting in more alkaline solutions because of the bicarbonate. This is an important reaction in controlling the amount of CO_2 in the atmosphere and can affect climate. Aluminosilicates when subjected to the hydrolysis reaction produce a secondary mineral rather than simply releasing cations.

$2\ KAlSi_3O_8 + 2\ H_2CO_3 + 9\ H_2O$ $Al_2Si_2O_5(OH)_4 + 4\ H_4SiO_4 + 2\ K^+ + 2\ HCO_3^-$

Orthoclase (aluminosilicate feldspar) + carbonic acid + water Kaolinite (a clay mineral) + silicic acid in solution + potassium and bicarbonate ions in solution

Biological weathering: A number of plants and animals may create chemical weathering through release of acidic compounds, i.e. the effect of moss growing on roofs is classed as weathering. Mineral weathering can also be initiated and/or accelerated by soil microorganisms. Lichens on rocks are thought to increase chemical weathering rates. For example, an experimental study on hornblende granite in New Jersey, USA, demonstrated a 3x – 4x increase in weathering rate under lichen covered surfaces compared to recently exposed bare rock surfaces. The most common forms of biological weathering are the release of chelating compounds (i.e. organic acids, siderophores) and of acidifying molecules (i.e. protons, organic acids) by plants so as to break down aluminium and iron containing compounds in the soils beneath them. Decaying remains of dead plants in soil may form organic acids which, when dissolved in water, cause chemical weathering. Extreme release of chelating compounds can easily affect surrounding rocks and soils, and may lead to podsolisation of soils. The symbiotic mycorrhizal fungi associated with tree root systems can release inorganic nutrients from minerals such as apatite or biotite and transfer these nutrients to the trees, thus contributing to tree nutrition. It was also recently evidenced that bacterial communities can impact mineral stability leading to the release of inorganic nutrients. To date a large range of bacterial strains or communities from diverse genera have been reported to be able to colonize mineral surfaces and/or to weather minerals, and for some of them a plant growth promoting effect was demonstrated.

Building weathering: Buildings made of any stone, brick or concrete are susceptible to the same weathering agents as any exposed rock surface. Alsostatues, monuments and ornamental stonework can be badly damaged by natural weathering processes. This is accelerated in areas severely affected by acid rain.

Properties of well-weathered soils

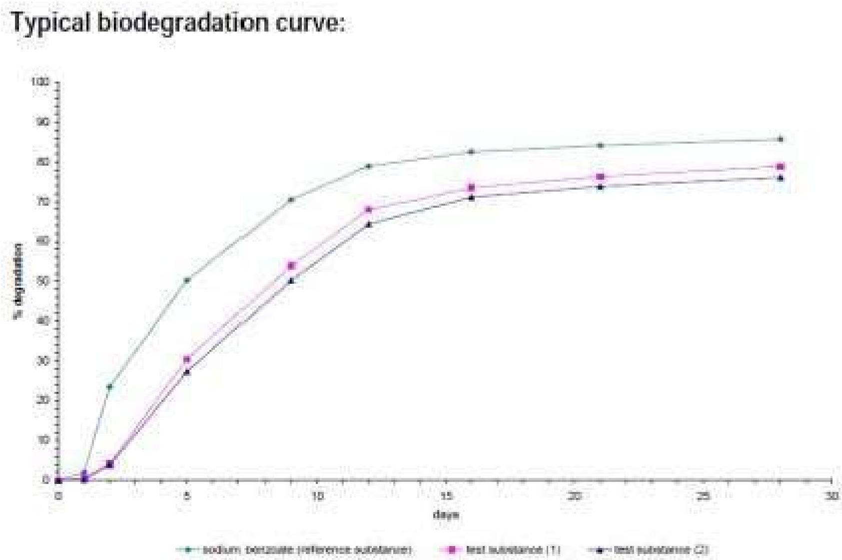
Three groups of minerals often remain in well-weathered soils: silicate clays, very resistant end products including iron and aluminium oxide clays, and very resistant primary minerals such as quartz. In highly weathered soils of humid tropical and subtropical regions, the oxides of iron and aluminium, and certain silicate clays with low Si/Al ratios, predominate because most other constituents have been broken down and removed.

Biodegradability test: Biodegradation data is essential to assess the fate and behaviour of substances in the environment. The ready or ultimate biodegradability of substances, e. g. detergent ingredients is therefore an important factor in ecological risk assessment. Biodegradation is a natural process carried out by microorganisms such as bacteria and fungi in combination with oxygen. Enzymatic processes lead to the degradation of the original compound and to the formation of smaller organic molecules. Some of these are used for production of biomass and others are converted to carbon dioxide, water and minerals (mineralisation). Biodegradation may be partial or total. Biodegradation tests measure the mineralisation of the organic carbon of the test substance under aerobic conditions. Depending on the test procedure, the test substance is dissolved in a mineral nutrient medium at a concentration of 10-40 mg/L carbon or 5-100 mg/L theoretical oxygen demand. The solution is inoculated with a small amount of microorganisms from a municipal waste water treating plant.

Mineralisation:

$$C_nH_{2n+2} \xrightarrow{\text{Microorganisms} + O_2} H_2O + CO_2$$

Typical biodegradation curve



It is then incubated for 28 days at 20 – 24 °C in the dark or in diffuse light. The biodegradation is followed at time intervals by measurement of oxygen depletion, CO2 evolution, the decrease of the content of dissolved organic carbon (DOC) or the decrease of chemical oxygen demand (COD). The incubation vessels are aerated with CO2-free air. The CO2 that is produced during biodegradation of the test substance is absorbed in gas wash bottles containing barium hydroxide solution. The amount of carbon dioxide produced is determined at intervals by replacing the barium hydroxide bottles with fresh ones and titrating the excess base. The course of biodegradation is shown graphically as a biodegradation curve.

Soil burial and compost conditions: Biodegradation occurs with enzymatic action and involves living organisms (micro/macro). Molecular degradation is promoted by enzymes and can occur under aerobic and anaerobic conditions, leading to complete or partial removal from the environment. Linear polymers are generally more biodegradable than branched polymers. The biodegradability of PHB has been examined in various environments such as in the soil, freshwater, and seawater. The rate of enzymatic degradation decreases with an increase in crystallinity crystal size and glass temperature. The enzymatic degradation rate of ductile materials is faster than in the case of perfectly crystalline and very crystalline material. Mixtures of PHB with poly (ε-caprolactone) PCL are biodegradable. The mixtures of PHB with both water soluble polyethylene oxide PEO and polyvinylalkohl PVA are biodegradable. PVAc is not water soluble, and is more slowly biodegradable than polyvinylalcohol. However, it changes to PVA by hydrolysis, and it is biodegradable like PVA. Doi and his co-workers have found out that PHB / 25 % PVAc is degradable. The enzymatic biodegradation of a material depends on the microbes, the pH-value, temperature, moisture, low glass temperature (high mobility), and low crystallinity. Anaerobic conditions do occur in garbage landfill or compost. The blends are degraded in the composting process by microbial bacteria via enzymes to carbon- dioxide and water. The degradation rates are, under composting conditions, higher than under natural conditions, because there is a higher temperature in the compost. The biodegradation of the blends' film was checked after 3 months on the compost heap. The samples were in the form of thin films, each being about 200 μm thick, which were prepared by hot pressing at 170°C and 50-bar pressure. On the upper-surface of the blend 10 film bacteria and fungi were found. The sample was washed with distillated water, and observed using Environment Scanning Electron microscopy ESEM. Figure (6.1 E, F) shows the surface of blend 5 films before and after degradation with a bio-film of hyphen, bacteria, and other microorganisms.

Biodegradability test: The surface of blend 11 was considerably eroded; only a few areas of original, non-degraded material were found (Figure 6.1). Pores of 1 - 5 μm had formed and the degradation caused a very rough topography, creating a larger surface area. The specimen was covered with dirt (EDX spectrum: Si, Al, Na, Mg, Cl, K, Ca from soil mineral) and soil microorganisms. In any case, in the densely populated areas the hyphen of fungi, bacteria and other things was found. There is evidence that microorganisms are able to enter larger pores (10 μm)

Test with river water: On the surfaces of specimens, stored in water, it is almost impossible to find uncovered, non-degraded areas. The surface is almost completely covered in organic and inorganic debris. The degraded surfaces, if visible, seem to undergo degradation, which is very similar to the degradation in the soil burial test. Pores were found having typical sizes of some microns. Degradation increases the surface area, helping the microorganisms to fix. The overall impression is that the degradation rate is higher than in soil burial tests. There are densely populated areas, where bacteria form a layer of organic material, completely covering the surface several unidentified microorganisms, including algae, were found.

Aerobic test: The aerobic biodegradability of organic compounds in an aqueous medium was evaluated by determining the oxygen requirement in a closed respirometer (Germany standard ISO 9408:1999). The aqueous medium contains a phosphate buffer in a water (5:1) solution. A bottles was filled with aqueous medium (250 ml) mixed with PHB or the blends (100 mg) and 10 mg microbial from sewage / sludge. We calculated the degradation rate = BOD / COD *100 from the biochemical oxygen demand (BOD) as a function of theoretical oxygen demand (ThOD) or chemical oxygen demand (COD) by using BSBdigi from Fa. Edmund Bühler. The degradation rate = BOD / COD *100 was calculated from the biochemical oxygen requirement (BOD) as a function of the theoretical oxygen requirement (ThOD) or chemical oxygen requirement (COD).

POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. What is bioremediation?
2. Comment on aquifer
3. What is eutrophication?
4. Comment on control devices of air pollution
5. What are the advantages of bioleaching?

PART-C (8 MARKS)

1. Give a detailed notes on causes and effects of air pollution
2. Write about the ill effect of air pollutions against human
3. Describe the microbial biodegradation
4. Explain the process of bioleaching
5. How to test the biodegradability

S.No	Unit III	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Which of the following microbe is widely used in the removal of industrial wastes	Trichoderma sp	Aspergillus niger	Pseudomonas putida	Bacillus	Aspergillus niger
2	______ normally absorb CO2 from atmosphere	Ocean	River	Lake	Pond	Ocean
3	_______ are mixed cultures of naturally occuring beneficial microbes used to degrade contaminants, increase quality of soil etc	Enhanced microorganisms	Efficient microorganisms	Effective microorganisms	Good microorganisms	Effective microorganisms
4	________ is a process of bioremediation used to improve quality of underground water	Bioaccumulation	Biostimulation	Bioventing	Biodegradation	Biostimulation and bioleaching
5	_________ bioremediation involves treating the contaminated material at the site	*Ex situ*	*In situ*	*In vivo*	*In vitro*	*In situ*
6	__________ is a key ingredient in organic farming	Compost	Urea	Pesticide	Fungicide	Compost
7	___________ involves the removal of the contaminated material to be treated elsewhere	*In vitro*	*In vivo*	*Ex situ*	*In situ*	*Ex situ*
8	___________ is a marine bacterium currently thought to be the world's most important oil-degrading organism	*Volvox globator*	*Zooglea ramigera*	*Chlorella pyrenoidosa*	*Alcanivorax borkumensis*	*Alcanivorax borkumensis*
9	___________ is the extraction of metals from their ores through the use of living organisms	Biofiltration	Bioleaching	Biobleaching	Biodegradation	Bioleaching
10	___________ is the world's largest known fossil water aquifer system					The Nubian Sandstone Aquifer System
11	____________ is an underground	Bore well	Ground well	Aquarium	Aquifers	Aquifers

	layer of water-bearing permeable rock, gravel, sand or silt					
12	______________ is a method of composting by piling biodegradable waste in long rows	Windrow method	Window method	Landfilling	In-vessel composting	Windrow method
13	A bacterium often used in hydrocarbon degradation	*Pseudomonas putida*	*Alcaligenes*	*Peptococcus*	*Sulfobacter*	*Pseudomonas putida*
14	A zone within the earth that restricts the flow of groundwater from one aquifer to another	Aquaguard	Aquitard	Atiquard	Quatitard	Aquitard
15	Acidification of ocean is due to absorbance of	Atmospheric O2	Atmospheric CO2	Atmospheric NO2	Atmospheric H2	Atmospheric CO2
16	Addition of archaea or bacterial cultures to speed up the rate of degradation of a contaminant	Bioaugumentation	Bioriching	Biobleaching	Biomediation	Bioaugumentation
17	An engineered device or system that supports a biologically active environment	Incubator	Shake flask	Biofilter	Bioreactor	Bioreactor
18	Bacteria used in bioleaching	*Vibrio cholerae*	*Salmonella typhi*	*Acidithiobacillus ferrooxidans*	*Lactobacillus caseii*	*Acidithiobacillus ferrooxidans*
19	Biostimulation is the process of	Organic farming	Enhancing growth conditions of microbes	Metal ore leaching	Isolation & Screening	Enhancing growth conditions of microbes
20	Carbofuran and parathion are	Herbicides	Fungicides	Pesticides	Weedicides	Pesticides
21	Common metal contaminants of sea	Magnesium	Aluminium	Copper	Zinc	copper
22	Earthworm species most often used are red wigglers that belongs to the genera	*Microcia*	*Epscicia*	*Eisenia*	*Bavaria*	*Eisenia*
23	Fungi that have the ability to degrade an extremely diverse range of toxic environmental pollutants	*Aspergillus niger*	*Mucor*	*Fusarium*	*Phanaerochaete chrysosporium*	*Phanaerochaete chrysosporium*
24	Greatest man-made river is in	Lebanon	Saudi arabia	Egypt	Libya	Libya
25	Intracellular accumulation of environmental pollutants such as	Bioriching	Biobleaching	Biomediation	Bioaccumulation	Bioaccumulation

	heavy metals by living organisms is					
26	Oldest form of waste treatment/disposal	Burning	Shredding	Vermicomposting	Landfill	Landfill
27	Process of supplying oxygen in situ to microbes in contaminated soil at low flow rates	Bioenhancing	Bioventing	Biomediation	Bioriching	Bioventing
28	Study of water flow in aquifers and the characterization of aquifers is called	Hydrogeology	Hydrology	Geology	Aqualogy	Hydrogeology
29	Technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site	Bioremediation	Disposal	Sewage treatment	Waste removal	Bioremediation
30	The process of composting using earthworms is called as	Degradation	Vermicomposting	Bioaugumentation	Organic treatment	Vermicomposting
31	The process of filtering water through a mass of roots to remove toxic substances or excess nutrients	Rhizofiltration	Microfiltration	Nanofiltration	Particulate filtration	Rhizofiltration
32	Which describes the treatment of environmental problems through the use of plants?	Bioremediation	Zooremediation	Phycoremediation	Phytoremediation	Phytoremediation
33	The use of living microorganism to degrade environment pollutants is called__________	microremediation	nanoremediation	bioremediation	all of these	bioremediation
34	Which of the following bacterium is called as the superbug that could clean up oil spills	Bacillus subtilis	Pseudomonas putida	Pseudomonas denitrificans	Bacillus denitrificans	Pseudomonas putida
35	The process of converting environmental polltants into harmless products by naturally occuring microbes is called __________	bioextraction	microbial extraction	biofiltration	bioleaching	bioleaching
36	Ex situ bioremediation involves the	Degradation of pollutants by microbes	Removal of pollutants and collection at a place to faxilitate	Degradation of pollutants by genetically	bioremediation	Removal of pollutants and collection at a place

		directly	microbial degradation			to facilitate microbial degradation
37	Which of the following is not an application of bioremediation	Treating oil spills by oil-eating microbes	Sewage treatment by bacteria	Burning of petroleum products floatng on the surface of the sea	Removal of grease by bacteris that digest FOG	Burning of petroleum products floatng on the surface of the sea
38	Which of the following is not true with respect to the advantages of bioremediation	Inexpensive	Cost effective	Contaminants are not completely destroyed	Environment friendly and natural process	Contaminants are not completely destroyed
39	Bioremediation is the process of removing contaminants or pollutants from the environment to return it to its original state through the use of _________ mechanisms.	Chemical	Abiotic	biological	Homeostatic	Biological
40	Process of using natural bacteria in mining industry is	Biodegradation	Biogenomics	Bioremediation	Bioleaching	Bioleaching
41	Term of biotechnology used to describe aquatic and marine applications is known as	blue biotechnology	Green biotechnology	White biotechnology	Red biotechnology	Blue biotechnology
42	The addition of known active microbes to soil or water with the purpose of accelerating microbial processes is called __________	Biodegradation	Bioremediation	Bioaccentuation	Bioaugmentatio n	Biodegradation
43	Which microorganisms are most commonly used bacteria for bioremediation?	Bacillus	Klebsiella	Staphylococcus	Streptococcus	Bacillus
44	Addition of archaea or bacterial cultures to speed up the rate of degradation of a contaminant	Bioaugumentati on	Bioriching	Biobleaching	Biomediation	Bioaugumentation
45	Which describes the treatment of environmental problems through the use of plants?	Bioremediation	Zooremediation	Phycoremediatio n	Phytoremediati on	Phytoremediation

46	Which of the following is not an application of bioremediation	Treating oil spills by oil-eating microbes	Sewage treatment by bacteria	Burning of petroleum products floatng on the surface of the sea	Removal of grease by bacteris that digest FOG	Burning of petroleum products floatng on the surface of the sea
47	Fungi that have the ability to degrade an extremely diverse range of toxic environmental pollutants	*Aspergillus niger*	*Mucor*	*Fusarium*	*Phanaerochaete chrysosporium*	*Phanaerochaete chrysosporium*
48	Bacteria used in bioleaching	*Vibrio cholerae*	*Salmonella typhi*	*Acidithiobacillus ferrooxidans*	*Lactobacillus caseii*	*Acidithiobacillus ferrooxidans*
49	Ex situ bioremediation involves the	Degradation of pollutants by microbes directly	Removal of pollutants and collection at a place to faxilitate microbial degradation	Degradation of pollutants by genetically	bioremediation	Removal of pollutants and collection at a place to facilitate microbial degradation
50	___________ is the world's largest known fossil water aquifer system					The Nubian Sandstone Aquifer System
51	The process of converting environmental polltants into harmless products by naturally occuring microbes is called _________	bioextraction	microbial extraction	biofiltration	bioleaching	bioleaching
52	Biostimulation is the process of	Organic farming	Enhancing growth conditions of microbes	Metal ore leaching	Isolation & Screening	Enhancing growth conditions of microbes
53	Oldest form of waste treatment/disposal	Burning	Shredding	Vermicomposting	Landfill	Landfill
54	_______ are mixed cultures of naturally occuring beneficial microbes used to degrade contaminants, increase quality of soil etc	Enhanced microorganisms	Efficient microorganisms	Effective microorganisms	Good microorganisms	Effective microorganisms
55	Intracellular accumulation of environmental pollutants such as	Bioriching	Biobleaching	Biomediation	Bioaccumulation	Bioaccumulation

	heavy metals by living organisms is					
56	Which of the following is not true with respect to the advantages of bioremediation	Inexpensive	Cost effective	Contaminants are not completely destroyed	Environment friendly and natural process	Contaminants are not completely destroyed
57	Greatest man-made river is in	Lebanon	Saudi arabia	Egypt	Libya	Libya
58	Bacteria used in bioleaching	*Vibrio cholerae*	*Salmonella typhi*	*Acidithiobacillus ferrooxidans*	*Lactobacillus caseii*	*Acidithiobacillus ferrooxidans*
59	Ex situ bioremediation involves the	Degradation of pollutants by microbes directly	Removal of pollutants and collection at a place to faxilitate microbial degradation	Degradation of pollutants by genetically	bioremediation	Removal of pollutants and collection at a place to facilitate microbial degradation
60	Carbofuran and parathion are	Herbicides	Fungicides	Pesticides	Weedicides	Pesticides

UNIT – IV

Biological nitrogen fixation - symbiotic and non-symbiotic microorganisms, root nodule formation, nitrogen fixers, hydrogenase, Nitrogenase, *Nif* gene regulation. Biochemistry of nitrogen fixation, Rhizosphere- R: S ratio, Interaction of microbes with plants. Bioconversion of agricultural wastes. Genetically Modified organisms and crops.

Biological Nitrogen Fixation: Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901 (Beijerinck 1901), is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Plants can readily assimilate NH_3 to produce the aforementioned nitrogenous biomolecules. These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as *Azotobacter*, bacteria that form associative relationships with plants, such as *Azospirillum*, and most importantly, bacteria, such as *Rhizobium*and *Bradyrhizobium*, that form symbioses with legumes

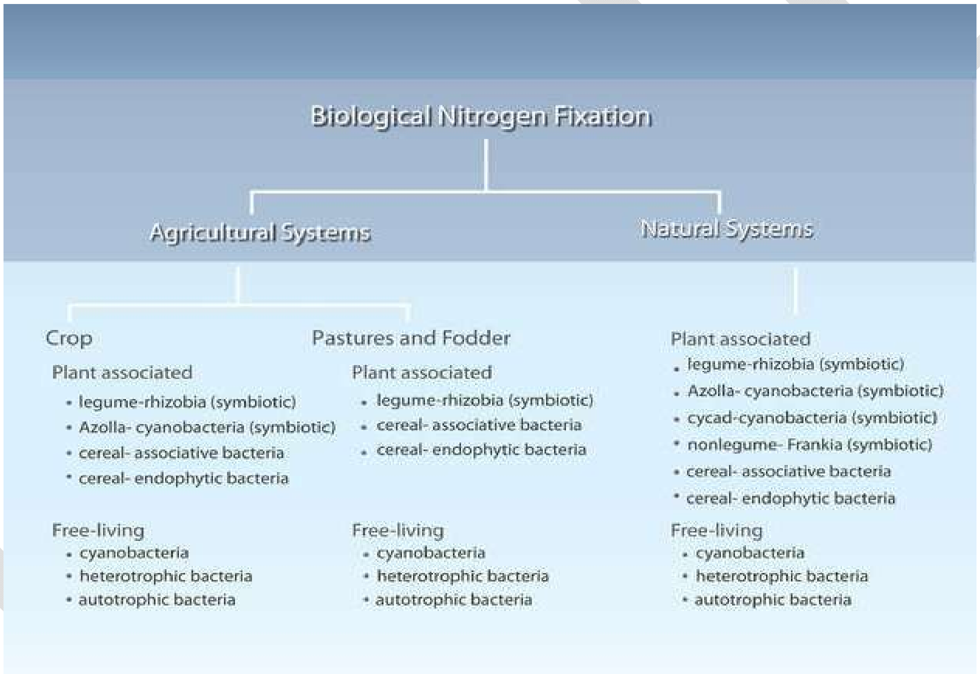


Table 1. Nitrogen-fixing organisms found in agricultural and natural systems.

Microorganisms that fix nitrogen require 16 moles of adenosine triphosphate (ATP) to reduce each mole of nitrogen. These organisms obtain this energy by oxidizing organic molecules. Non-photosynthetic free-living microorganisms must obtain these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars produced by photosynthesis. Associative and symbiotic nitrogen-fixing microorganisms obtain these compounds from their host plants' rhizospheres

Two kinds of nitrogen fixers are recognized: free-living (non-symbiotic) bacteria, including the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and such genera as *Azotobacter,* *Beijerinckia,* and *Clostridium*; and mutualistic (symbiotic) bacteria such

as *Rhizobium,* associated with leguminous plants, and *Spirillum lipoferum,* associated with cereal grasses.

The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules the bacteria convert free nitrogen to nitrates, which the host plant utilizes for its development. To insure sufficient nodule formation and optimum growth of legumes (*e.g.,* alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

Nitrogen Fixation by Free-Living Heterotrophs: Many heterotrophic bacteria live in the soil and fix significant levels of nitrogen without the direct interaction with other organisms. Examples of this type of nitrogen-fixing bacteria include species of *Azotobacter*, *Bacillus*, *Clostridium*, and *Klebsiella*. As previously noted, these organisms must find their own source of energy, typically by oxidizing organic molecules released by other organisms or from decomposition. There are some free-living organisms that have chemolithotrophic capabilities and can thereby utilize inorganic compounds as a source of energy. Because nitrogenase can be inhibited by oxygen, free-living organisms behave as anaerobes or microaerophiles while fixing nitrogen. Because of the scarcity of suitable carbon and energy sources for these organisms, their contribution to global nitrogen fixation rates is generally considered minor. However, a recent study in Australia of an intensive wheat rotation farming system demonstrated that free-living microorganisms contributed 20 kilograms per hectare per year to the long-term nitrogen needs of this cropping system (30-50% of the total needs. Maintaining wheat stubble and reduced tillage in this system provided the necessary high-carbon, low-nitrogen environment to optimize activity of the free-living organisms.

Associative Nitrogen Fixation: Species of *Azospirillum* are able to form close associations with several members of the *Poaceae* (grasses), including agronomically important cereal crops, such as rice, wheat, corn, oats, and barley. These bacteria fix appreciable amounts of nitrogen within the rhizosphere of the host plants. Efficiencies of 52 mg N_2 g^{-1} malate have been reported (Stephan *et al*. 1979). The level of nitrogen fixation is determined by several factors, including soil temperature (*Azospirillum* species thrive in more temperate and/or tropical environments), the ability of the host plant to provide a rhizosphere environment low in oxygen pressure, the availability of host photosynthates for the bacteria, the competitiveness of the bacteria, and the efficiency of nitrogenase

Symbiotic Nitrogen Fixation: Many microorganisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilized by the nitrogen-fixing microorganism for the energy it needs for nitrogen fixation. In exchange for these carbon sources, the microbe provides fixed nitrogen to the host plant for its growth.

Example of this type of nitrogen fixation is the water fern *Azolla*'s symbiosis with a cyanobacterium *Anabaena azollae*. *Anabaena* colonizes cavities formed at the base of *Azolla* fronds. There the cyanobacteria fix significant amounts of nitrogen in specialized cells called heterocysts. This symbiosis has been used for at least 1000 years as a biofertilizer in wetland paddies in Southeast Asia. Rice paddies are typically covered with *Azolla*–blooms that fix up to 600 Kg N ha^{-1} yr^{-1} during the growing season. Another example is the symbiosis between actinorhizal trees and shrubs, such as Alder (*Alnus* sp.), with the actinomycete *Frankia*. These plants are native to North America and tend to thrive in nitrogen-poor environments. In many areas they are the most common non-legume nitrogen fixers and are often the pioneer species in successional plant communities. Actinorhizal plants are found in many ecosystems including alpine, xeric, chapparal, forest, glacial till, riparian, coastal dune, and arctic tundra environments.

Even though the symbiotic partners described above play an important role in the worldwide ecology of nitrogen fixation, by far the most important nitrogen-fixing symbiotic associations are the relationships between legumes and *Rhizobium* and *Bradyrhizobium* bacteria. Important legumes used in agricultural systems include alfalfa, beans, clover, cowpeas, lupines, peanut, soybean, and vetches. Of the legumes in agricultural production, soybeans are grown on 50% of the global area devoted to legumes, and represent 68% of the total global legume production.

2. Symbiotic micro organisms

Bacterial Symbiosis: Bacteria form symbiotic relationships with many organisms, including humans. One example is the bacteria that live inside the human digestive system. These microbes break down food and produce vitamins that humans need. In return, the bacteria benefit from the stable environment inside the intestines. Bacteria also colonize human skin. The bacteria obtain nutrients from the surface of the skin, while providing people with protection against more dangerous microbes.

Symbiotic bacteria also live in nodules on the roots of bean plants. These bacteria convert nitrogen gas into a form that the plants can use. In return, the plants provide the bacteria with a safe place to live. In some cases, the symbiotic relationship is more strong. One particular roundworm has a bacterium living inside it. The roundworm infects and kills insects, using a toxin produced by the bacterium. This is an example of mutualism, because the roundworm and bacterium need each other to survive.

Fungi and Plants: Fungi and plants form mutually-beneficial relationships called mycorrhizal associations. The fungi increase the absorption of water and nutrients by the plants, and benefit from the compounds produced by the plants during photosynthesis. The fungus also protects the roots from diseases. Some fungi form extensive networks beneath the ground, and have been known to transport nutrients between plants and trees in different locations.

Fungi and plant roots form two different kinds of associations. In one type, the fungus grows outside the roots as a thick mat, or between certain cells in the root. The fungus, however, never enters any of the plant cells. With the other type of association, the fungus actually penetrates the cell walls of the roots. They break through the cell wall, but not the inner plasma membrane. In both types, the fungus extends its filaments outward to collect nutrients and water from the soil, which are in turn passed onto the plant.

Lichens: Fungi and Algae

Lichens are an example of a symbiotic relationship between two microbes, fungi and algae. So far, around 25,000 lichens have been identified. They grow on rocks and tree trunks, with colors ranging from pale whitish green to bright red and orange. The lichens grow in several forms: thin and crusty coverings; small branching strands; or flat, leaf-like structures. They are usually the first plants to grow in the cold and dry habitats that they favor.

In this mutually-beneficial relationship, the fungus forms the body of the lichen — the thallus. This structure attaches to the surface of a rock or tree. The fungal cells absorb water and nutrients from the surrounding environment. Algal cells grow inside the cells of the fungus. The algal cells convert sunlight to chemical energy through photosynthesis. This process benefits the fungus. In return, the algal cells are protected from the environment.

Protists : Certain protists and algae form a symbiotic relationship known as living sands. This type of association occurs in tropical and semitropical seas, and appears as green, orange, brown or red deposits containing calcium carbonate. Living sands were used in the construction of the Egyptian pyramids. Many different types of algae combine with their protist hosts. Without the algae, the protists cannot survive very long. Similar to living sands, some protists extract chloroplasts from diatoms, a type of algae. The chloroplasts provide the protists with the ability to convert sunlight to chemical energy through photosynthesis. Eventually, the chloroplasts break down and stop functioning.

An even better-known example of symbiotic protists are the ones that live in the guts of termites. These microbes break down cellulose in the wood particles that termites eat. This enables the termite to obtain nutrition from the wood. Without the help of the protists, the termite would not be able to digest the wood. In this case, the protist is called an endosymbiont, which means it lives inside its host, the termite.

Early Eukaryotes: Some scientists believe that early in the history of the planet, different types of microbes joined together to form a new type of organism. At the time, certain bacteria had the ability to convert sunlight to chemical energy, or generate chemical energy from oxygen. These microbes were engulfed by larger bacteria, forming a microbial symbiosis. The host cell protected the smaller microbe inside, while benefitting from the skills of its new partner.

In the beginning, the two bacteria could still function separately. Eventually, the microbes living inside lost the ability to survive on their own, and they became specialized components of the host cells. These structures later became the mitochondria and chloroplasts of eukaryotic cells. Mitochondria generate energy using oxygen, and chloroplasts convert sunlight into chemical energy in plant cells. Supporting this theory is the fact that mitochondria and chloroplasts both have their own DNA, separate than that found in the nucleus of the cell.

3. Non-symbiotic Nitrogen fixers

Azospirillum : Eighty percent (80 %) of the atmosphere is nitrogen gas (N2). Unfortunately N2 is unusable by most living organisms. Plants, animals, and microorganisms can die of nitrogen deficiency, if surrounded by N_2 they cannot use. All organisms use the ammonia (NH_3) form of nitrogen to manufacture amino acids, proteins, nucleic acids, and other nitrogen-containing components necessary for life. Biological nitrogen fixation (BNF) process changes inert N_2 to useful NH_3. This process is mediated in nature only by bacteria and certain species of actinomycetes. In the free-living system, plants gain benefit when the bacteria die and release nitrogen to the environment, or when the bacteria are loosely associated with the roots of plants. In legumes and a few other plants, the bacteria live in small club-like growths on the roots called nodules. Within these nodules, N_2 fixation occurs, and the NH_3 produced is directly absorbed by the plant. Nitrogen fixation by legumes is a close/symbiotic relationship between a Rhizobium bacterium and a legume host plant. Azospirillum as a ―biofertilizer‖ is particularly important in agricultural systems where fertilizer inputs are either impractical (rangelands), undesirable (organic farming), or not possible (subsistence agriculture). Experiments on inoculation of crops with Azospirillum or other diazotrophs resulted in enhanced plant growth or nitrogen content under environmental conditions, improve nutrient assimilation, alter root size and function. Numerous studies have shown greater N_2 fixation activities in inoculated plants than in uninoculated controls. The report shows higher N_2 fixation rates were observed near or at flowering stage particularly under conditions of high temperature and soil moisture. In addition to N_2 fixation, inoculation with Azospirillum results in the following benefits:

1. Promotion of root hair development and branching;
2. Increased uptake of N, P, K and microelements;
3. Improved water status of plants and,
4. Increased dry matter accumulation and grain yield.

Inoculated plants when examined under the electron microscopes revealed invasion of the cortical layer. Azospirillum species are described as Gram negative, rod-shaped, 1mm in diameter, very motile. Cells are about 1.0 um x 3.5 mm in size single flagellum when grown in MPSS broth while lateral flagella when grown on MPSS agar at 30 ºC. They also form wrinkled, dark pink colonies when grown on MPSS agar. A formation of a white veil or bacteria band, is visible when inoculated into an Nfb and Dobereiner's liquid medium. Azospirillum utilizes glucose, lactate, succinate, fructose, malate, pyruvate, fumarate, as carbon source, reduced nitrate and does not require biotin. The N source used by Azospirillum for their growth**: 1.** Ammonium **2.**Nitrate **3.**Amino acids **4.**Elemental N. *Azospirillum spp*. are highly adaptable, being able to grow under:

1.Anaerobic conditions (nitrate used as eletron acceptor) **2.**Microaerobic (elemental or ammonia used as N source) **3.**Fully aerobic conditions (ammonia, nitrate, amino acid or combined N only) Preliminary field experiments in Batangas, Pangasinan, Laguna, Bulacan and Cagayan Valley showed when BIO-N inoculated corn produce a comparatively high yield in the presence of 1/3 to 2/3 of the required N fertilizer. In most of the test sites, the inoculated but unfertilized plots gave rise to consistently and significantly taller and greener plants than the uninoculated unfertilized control, particularly at sixty days after planting.

4. Legume Nodule Formation

The *Rhizobium* or *Bradyrhizobium* bacteria colonize the host plant's root system and cause the roots to form nodules to house the bacteria. The bacteria then begin to fix the nitrogen required by the plant. Access to the fixed nitrogen allows the plant to produce leaves fortified with nitrogen that can be recycled throughout the plant. This allows the plant to increase photosynthetic capacity, which in turn yields nitrogen-rich seed. The consequences of legumes not being nodulated can be quite dramatic, especially when the plants are grown in nitrogen-poor soil. The resulting plants are typically chlorotic, low in nitrogen content, and yield very little seed.

The nodulation process illustrates an orchestrated interaction between the bacteria and host plant. The process begins when the rhizobia are attracted to flavonoids released by the host legume's roots. For legumes like alfalfa, clover, and soybeans (others like lupines and peanuts form nodules in other ways) the bacteria then begin to attach themselves to extensions of root epidermal cells called root hairs. The attachment process is actually a two-step process where the bacteria first attach using a Ca^{2+} - binding protein called rhicadhesin. After the bacteria accumulate and anchor themselves to the root hair surface, a firmer attachment that involves lectins and/or cellulose firbrils and fimbriae produced by the host plant and bacteria, respectively.

The host legume then senses chemicals produced by the rhizobia called Nod factors that cause the colonized root hairs to curl and form what is called a shepherd's crook. Then rhizobia penetrate the root hairs and typically form a tubular structure called an infection thread. Once the bacteria reach the root itself, they stimulate cortical cell divisions that lead to the formation of a nodule. As the nodule begins to form, the bacteria become surrounded by a plant-derived membrane and are released inside plant cells forming the nodule. The bacteria subsequently lose their cell walls and undergo a profound change in cell morphology to form large, irregularly shaped branching cells called bacteroids. They then are entirely dependent on the host plant for their energy needs. In return, the bacteria fix nitrogen for the plant.

The interaction between the bacteria and host legume is so intricate that a particular *Rhizobium* or *Bradyrhizobium* will only nodulate a select number of plant genera. For example, *Rhizobium melilotii* will only nodulate alfalfa, while *Rhizobium leguminosarum biovar trifolii* will only nodulate clover (*Trifolium*). This host specificity is referred to cross inoculation group cell signaling between the bacteria and the legume host. The aforementioned Nod factors have been identified as lipochitin oligosaccharides. Variations in the structures of these oligosaccharides determine the host specificity for the bacterium.

Nitrogen is an essential nutrient for plant growth and development but is unavailable in its most prevalent form as atmospheric nitrogen. Plants instead depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide, ecological problems, such as the formation of coastal dead zones. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. It is a critical component of many aquatic, as well as terrestrial ecosystems across our biosphere.

5. Nitrogen fixers and Hydrogenase enzyme: zotobacter possesses an active hydrogenase, the enzyme which catalyzes the oxidation of hydrogen. Some evidence has been uncovered which suggests that the occurrence of this enzyme is correlated with nitrogen fixation by this organism. If true, this has great significance for studies of the mechanism of biological nitrogen fixation. A detailed investigation was accordingly undertaken and it was measured the hydrogenase activity of Azotobacter cells grown or maintained under conditions which would vary the rate or extent of the nitrogen-fixing reaction. These included (a) the use of various forms of combined nitrogen; (b) variation in the level of combined nitrogen; (c) adaptation of cells to combined nitrogen; (d) growing cells in the presence of hydrogen and absence of free nitrogen. The general methods have been described in earlier reports; any necessary details will be furnished in the text.

Effect of pH of Medium on Hydrogenase in Axotobacter: In the initial experiments with combined nitrogen (2) ammonium phosphate usually served as a source of this element. During growth a pronounced decrease in the pH occurred because of selective utilization of the ammonium ion. however, it is the presence of combined nitrogen in the medium and not the alteration of pH which is accompanied by a decrease in hydrogenase. The data demonstrate that the hydrogenase con- tent of Axotobacter drops in the presence of ammonium ion, whet,her the pH rises, falls, or remains constant.

Effect of Combined Nitrogen on Hydrogenase in Various Species of Azotobacter-Cultures of *Axotobacter vinelandii*, *A. agile*, and *,4. chroococcum* were grown on Burk's N-free agar medium and on the same medium plus combined nitrogen in various forms (50 mg. of N per 100 ml.). Table II gives the results of these experiments. As noted previously with *A. vinelandii* (2), NHk-N is much more effective in reducing the hydrogenase content than is NOS-N, whereas glutamate N has little if any effect.

6. Nitrogenase

Nitrogenase and its metalloclusters : Diazotrophic organisms can fix N2 because they produce an enzyme called nitrogenase. Most N2 fixing organisms studied so far produce a Molybdenum-containing 2 nitrogenase. In addition, some organisms have ―alternative‖ systems that produce a Vanadium-containing nitrogenase and/or an iron-only nitrogenase. Among these three classes of nitrogenase, the Mo-containing nitrogenase is the most prevalent and the best characterized. Mo-containing nitrogenases are composed of two oxygen – sensitive components designated the MoFe protein and the Fe protein. Together under the ideal conditions, they catalyze the following reaction:

$$N_2 + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

7. Nif gene regulation

Fig:3

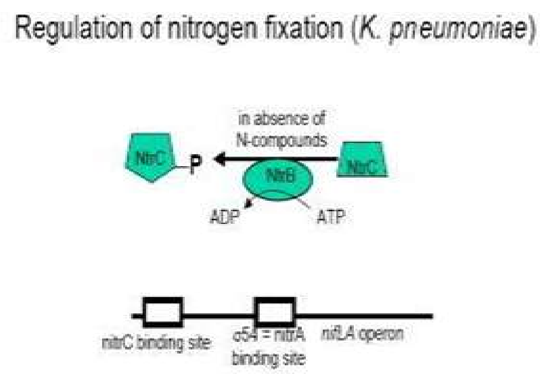
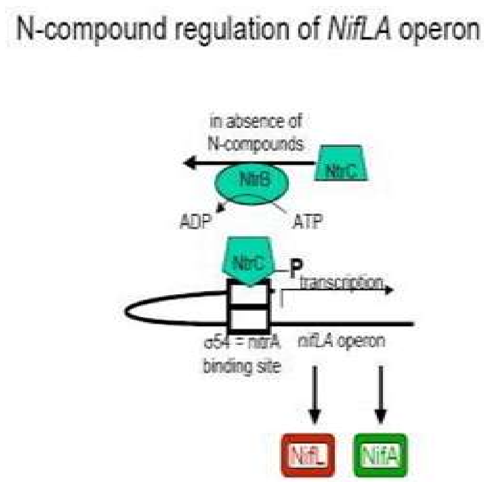


Fig:4



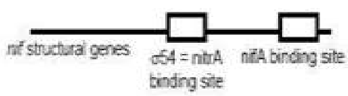


Fig:5

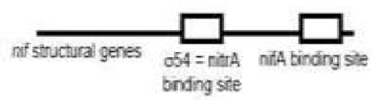


Fig:6

8. Biochemistry of Nitrogen fixation: To understand the biosynthesis of the iron-molybdenum cofactor of nitrogenase **(FeMo-co; Figure 7**) and to improve biological hydrogen production by altering the catalytic properties of nitrogenase. Our future research plans include applying the knowledge obtained through basic science studies of nitrogenase biogenesis to engineer active nitrogenase in eukaryotes.

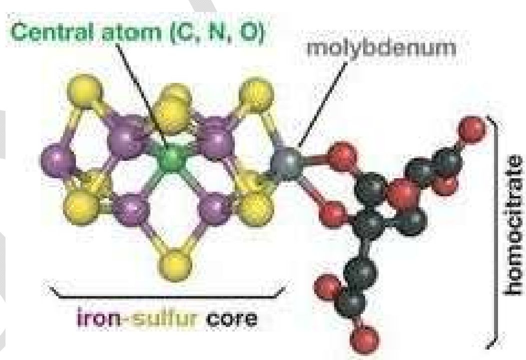
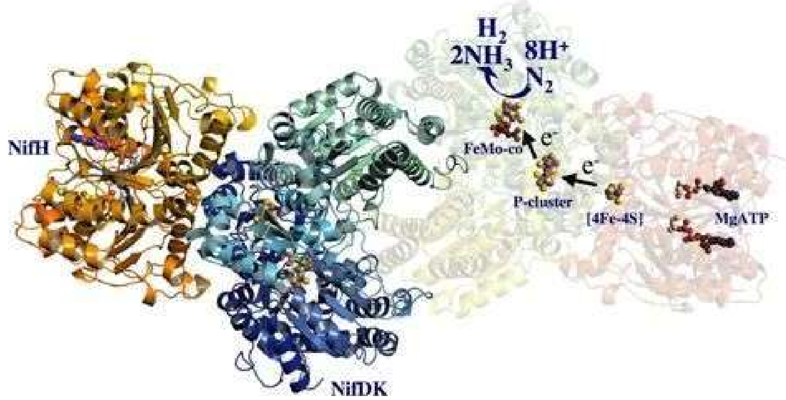


Fig: 7 Structure of the nitrogenase enzyme complex

FeMo-co, located at the active site of the nitrogenase enzyme is utimately responsible for biological nitrogen fixation, a process that transforms inert atmospheric N2 into a form that can be metabolized by organisms **(Figure 8).** Although only a small group of bacteria and archea are able to fix nitrogen, this essential natural process supports life on earth. Due to its great commercial (agricultural) significance, nitrogenase has been subjected to extensive biochemical, genetic, and structural analyses. Understanding the details of FeMo-co synthesis and nitrogenase assembly could, in the long term, improve the agronomical applications of biological nitrogen fixation.



$$N_2 + 8H^+ + 16MgATP + 8e^- \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$$

Fig:8 Structure of the Nitrogenase enzyme complex

9.Rhizosphere-R:S ratio

The root system of higher plants is associated not only with soil environment composed of inorganic and organic matter, but also with a vast community of metabolically active microorganisms. As living plants create a unique habitat around the roots, the microbial population on and around the roots is considerably higher than that of root free soil environment and the differences may be both quantitative and qualitative.

1. **Rhizosphere:** It is the zone/region of soil immediately surrounding the plant roots together with root surfaces, or it is the region where soil and plant roots make contact, or it is the soil region subjected to influence of plant roots and characterized by increased microbial.

2. **Rhizoplane:** Root surface along with the closely adhering soil particles is termed as rhizoplane.

Microorganisms in the Rhizosphere and Rhizosphere Effect

The rhizosphere region is a highly favorable habitat for the proliferation, activity and metabolism of numerous microorganisms. The rhizosphere microflora can be enumerated intensively by microscopic, cultural and biochemical techniques. Microscopic techniques reveal the types of organisms present and their physical association with the outer root tissue surface / root hairs. The cultural technique most commonly followed is "serial dilution and plate count method" which reveal the quantitative and qualitative population of microflora. At the same time, a cultural method shows the selective enhancement of certain categories of bacteria. The biochemical techniques used are designed to measure a specific change brought about by the plant or by the microflora. The rhizosphere effect on most commonly found microorganisms viz. bacteria, actinomycetes, fungi, algae and protozoa is being discussed herewith in the following paragraphs.

A.Bacteria: The greater rhizosphere effect is observed with bacteria (R: S values ranging from 10-20 or more) than with actinomycetes and fungi. Gram-negative, rod shaped, non-sporulating bacteria which respond to root exudates are predominant in the rhizosphere*(Pseudomonas, Agrobacterium).* While Gram-positive, rods, Cocci and aerobic spore forming *(Bacillus, Clostridium)* are comparatively rare in the rhizosphere. The most common genera of bacteria are: *Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* and others have been reported to be either abundant or sparse in the rhizosphere. From the agronomic point of view, the abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere assumes a great importance. The aerobic bacteria are relatively less in the rhizosphere because of the reduced oxygen levels due to root respiration. The bacterial population in the rhizosphere is enormous in the ranging form 10^8 to 10^9 per gram of rhizosphere soil. They cover about 4-10% of the total root area occurring profusely on the root hair region and rarely in the root tips. There is predominance of amino acids and growth factors required by bacteria, are readily provided by the root exudates in the region of rhizosphere.

B.Fungi: In contrast to their effects on bacteria, plant roots do not alter / enhance the total count of fungi in the rhizosphere. However, rhizosphere effect is selective and significant on specific fungal genera *(Fusarium, Verticillium, Aspergillus* and *Penicillium)* which are stimulated. The R:S ratio of fungal population is believed to be narrow in most of the plants, usually not exceeding to 10. The soil / serial dilution and plating technique used for the enumeration of rhizosphere fungi may often give erratic results as most of the spore formers produce abundant colonies in culture media giving a wrong picture / estimate *(eg Aspergilli* and *Penicillia).* In fact the mycelial forms are more dominant in the field. The zoospore / forming lower fungi such as *Phytophthora, Pythium, Aphanomyces* are strongly attracted to the roots in response to particular chemical compounds excreted by the roots and cause diseases under favorable conditions. Several fungi *eg Gibberella* and *fujikurio*produces *phytohormones* and influence the plant growth.

C.Actinomycetes, Protozoa and Algae: Stimulation of actinomycetes in the rhizosphere has not been studied in much detail so far. It is generally understood that the actinomycetes are less stimulated in the rhizosphere than bacteria. However, when antagonistic actinomycetes increase in number they suppress bacteria. Actinomycetes may also increase in number when antibacterial agents are sprayed on the crop. Among the actinomycete, the phosphate solublizers (eg.*Nocardia, Streptomyces)* have a dominant role to play.

As rule actinomycetes, protozoa and algae are not significantly influenced by their proximity to the plant roots and their R: S ratios rarely exceed 2 to 3: 1 and around roots of plants, R: S ratio for these microorganisms may go to high. Because of large bacterial community, an increase in the number or activity of protozoa is expected in the rhizosphere. Flagellates and amoebae are dominant and ciliates are rare in the region.

Table:3 Factors Responsible for the Development of the Soil-Plant Root Rhizosphere.

Release of soluble organic compounds by plant roots
Sloughed off root cell debris and dying root hairs
Plant root cell lysis
Higher concentration of carbon dioxide
Lower concentration of oxygen
Lower concentration of nutrient ions
Partial desiccation of soil due to absorption of water by roots

10. Interaction of microbes with plants: Plants are in contact with diverse microbes blown by the wind, delivered via the water cycle, and recruited to their roots and leaves from the soil. Many of these microbes are unable to start their life cycle in association with a living plant. Others are potential pathogens, potential symbionts, or harmless commensals. The ultimate outcome of plant-microbe interactions is tuned by host and microbe genotypes and by the environmental context. All land plants grow in intimate association with complex microbial communities. These attach to, and inhabit, both the roots (rhizosphere) and aboveground organs (phyllosphere) as epiphytes or endophytes. Plant-derived exudates and secreted secondary metabolites are implicated in encouraging specific microbial colonization. Host plants often rely on the associated microbiome for one or more critical nutrients, such as fixed nitrogen. The plant, in turn, can provide fixed atmospheric carbon to some members of the microbiome, thus acting as a carbon sequestration niche.

Plant interactions with microbes are important in the context of plant health and global food security. Yield losses due to microbial pathogens and pests can be up to 30 percent worldwide, and much of this loss takes place after the freshwater input required to grow a crop. Thus, if we could better combat microbial infection of plants via rational deployment of the plant immune system, we could save significant amounts of water and spare significant acreage from the plow.

Additionally, if we could better understand and deploy the plant immune system, we could diminish or eliminate the use of chemicals in the control of plant disease.

The Plant Immune System: Plants express a two-tiered immune system that has analogies to the mammalian innate immune system. Microbes express microbial-associated molecular patterns (MAMPs) on their surfaces. MAMPs can be sensed specifically by plant cell-surface pattern-recognition receptors (PRRs). Plant PRRs described to date are cell-surface receptors featuring an ectodomain, most commonly a leucine-rich repeat (LRR), a transmembrane domain, and a cytosolic kinase domain. Plant PRRs are analogous to the familiar TLR receptors of the animal innate immune system. MAMP recognition leads to signal transduction and transcriptional reprogramming, resulting in the initiation of MAMP-triggered immunity (MTI). MTI is sufficient to halt the growth of most microbes. Hence, most microbes are not pathogens.

The Plant Microbiome: All land plants grow in intimate association with a complex root microbiota that is distinct from the microbial community present in bulk soil. These interactions are driven by the influence of root physiology and metabolism, which influence the rhizoplane (the 1 mm surrounding the root) environment through adjusting the soil pH changing soil structure and oxygen availability, producing antimicrobials and quorum-sensing mimics that manipulate microbial communication, providing an energy source in the form of dead root material and carbon-rich exudates, and more. In fact, between 5 and 33 percent of fixed atmospheric carbon is sequestered in the rhizosphere. The microbial communities that inhabit this niche can have a net beneficial or net detrimental impact on plant health, and shifting this balance is of major agronomic interest. Various mutualistic rhizosphere microbes provide the host plant with physiologically accessible nutrients; improve plant growth through production of phytohormones; help plants withstand heat, salt, and drought; act as protectants against phytopathogens; and more.

Microbial community structure differs across plant species and also among some inbred genotypes within single species grown in a common soil. Studies abound in a variety of systems to address the host genetic effect on the microbiome for various crops and other plants. Rhizosphere and microbial community analysis studies in *Arabidopsis* are also becoming more common, although these lack power because they use methods that are not readily comparable among studies, use low-resolution phylotyping techniques, and suffer from small sample sizes.

To define a robust system in which host genes important in shaping definable microbial phenotypes could be subsequently identified. Such host genes would constitute practical targets for intervention in crop plants to promote plant health in particular soil and climate conditions. One critical question is whether the gain and loss of effectors, and their subsequent recognition, play a role in the evolution or interconversion of pathogens and mutualists. Another is to what degree the known components of the plant immune system modulate the assemblage of the root microbiome. These new projects will benefit from integration with our ongoing studies of both effector diversity and plant immune function.

Using *Arabidopsis thaliana* inbreds and related Brassicaceae, we applied multiplexed pyrosequencing of microbial 16S rRNA genes from the root systems of hundreds of individual plants to test the hypothesis that the microbiota of a plant grown in wild soils is sufficiently dependent on host genotype to vary among related inbred individuals. We found distinct microbial communities in bulk soil, rhizosphere, and endophyte fractions that are influenced by soil type and plant developmental stage. We also found that plant genotype directs the assembly of robust microbial phenotypes, setting the stage for genetic dissection of responsible host loci.

11. Bioconversion of Agricultural wastes

2.1. Solid-State Fermentation for Bioconversion of Agricultural and Food Processing Waste into Value-Added Products

Solid-state fermentation (SSF), a general method for food processing waste bioconversion, is a process in which microorganisms grow on or within solid substrates in the absence of free water. However, substrates must possess enough moisture to support the growth and metabolism of microorganisms (56). The solid material in this process acts both as physical support and source of nutrients. To simplify product isolation from the medium, for example, polyurethane foam may be used instead of natural raw material such as wheat bran. SSF has been conventionally more applicable for filamentous fungi, but yeast and even bacteria are successfully used for biotechnological production by solid-state fermentation. SSF is a low-level technology in comparison with industrial submerged fermentation, but it appears to be a promising technology for the utilization of solid wastes. SSF is of special interest to countries with an abundance of agro-industrial residues that can be used as inexpensive raw materials. SSF has many advantages in processing agro-industrial residues as compared with submerged fermentation: lower energy requirements, process simplicity, cheaper aeration, absence of rigorous control of fermentation parameters and production of smaller quantity of

Bioconversion of food processing waste in products

Waste	Value-added product	Microorganism
Apple pomace, cranberry pomace and strawberry pomace	Enzyme Polygalacturonase	*Lentinus edodes*
Wheat bran, sunflower flour, coffee husk, soybean meal, rice bran, corn bran, rice hull, aspen wood, sweet potato residue, waste hair	Enzymes Proteases (acidic, neutral and alkaline)	*Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Bacillus* sp., *Trichoderma* sp.
The mixture of sugar cane bagasse with wheat bran or orange bagasse	Enzyme Pectin lyase and polygalacturonase	*Thermoascus aurantiacus*
Lemon pulp	Enzyme Pectinase	*Trichoderma viride*
Wheat bran, rice bran, apple pomace	Enzyme Pectinase	*Bacillus* sp.
Wheat bran, rice bran, coconut oil cake and corn flour	Enzyme Inulinase	*Staphylococcus* sp., *Kluyveromyces marxianus*
Lignocellulosic wastes, sugarcane bagasse	Enzymes Cellulase β-glucosidase	*Aspergillus ellipticus*, *Aspergillus fumigatus*
Banana waste	Enzymes α-amylase Cellulases	*Bacillus subtilis*
Tea waste	Enzyme Glucoamylase	*Aspergillus niger*

Value-Added Biotechnological Products from Organic Wastes

Table 8.3
(Continued)

Pineapple waste	Organic acid Citric acid	*Aspergillus foetidus* *Aspergillus niger*
Sugarcane bagasse	Amino acid L-glutamic acid	*Brevibacterium* sp.
Sweet potato residue	Antibiotic Tetracycline Chlorotetracycline	*S. viridifaciens*
Soybean curd residue, okara	Antibiotic Iturin A	*Bacillus subtilis*
Wheat bran	Plant growth hormone Gibberellic acid	*Gibberella fujikuroi*
Cassava flour, sugar cane bagasse	Plant growth hormone Gibberellic acid	*Gibberella fujikuroi*
Hydrolyzed tomato pomace	Vitamin B_{12}	*Propionibacterium shermanii*
Prawn-shell waste	Single cell protein	*Candida* spp. *Rhodotorula* spp.
Olive pomace after delignification and saccharification	Poultry feed enriched by protein	*Candida utilis* or *Saccharomyces cerevisiae*
Apple pomace	Animal feed	*Aspergillus niger* and

Hydrolyzed potato starch waste	Exopolysaccharide Pullulan	*Aureobasidium pullulans*
Grape skin pulp extract, starch waste, olive oil waste effluents, molasses	Exopolysaccharide Pullulan	*Aureobasidium pullulans*
Sent malt grains, apple pomace, grape pomace, and citrus peels	Exopolysaccharide Xanthan	*Xanthomonas campestris*
Olive mill wastewaters	Exopolysaccharide Xanthan	*Xanthomonas campestris*
Coconut waste	Bacterial endotoxins	*Bacillus thuringiensis*

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Comment on biological nitrogen fixation
2. What is symbiosis
3. What is role of *Nif* gene
4. What is rhizosphere and rhizoplane
5. Comment on the importance of beneficial microbes for crop improvements

PART-C (8 MARKS)

1. Describe the genetically modified organisms
2. Explain the role of symbiotic microbes to biological nitrogen fixation
3. Give a detailed notes about the microbial conversion of agricultural wastes
4. Comment on the biochemistry of nitrogen fixation
5. Describe the microbial interaction with plants

S.No	Unit IV	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	______ are aerobic and free-living nitrogen nitrogen fixers	Frankia & Azospirillum	*Clostridium& Desulfovibrio*	Beijerinckia & Klebsiella	*Rhizobium& Anabaena*	Beijerinckia & Klebsiella
2	______ are genes encoding enzymes involved in the fixation of atmospheric nitrogen	*mif*	*nif*	*sif*	*nod*	*nif*
3	________ catalyze conversion of atmospheric nitrogen to ammonia	Kinase	Hydrogenase	Nitrogenase	Phosphatase	Nitrogenase
4	________ is a typical example of symbiotic nitrogen fixation seen in paddy fields	Azolla-Anabaena	Alder-Frankia	Legume-Rhizobium	Higher plants-Mycorrhizae	Azolla-Anabaena
5	_________ recycles the H2 produced during N2 fixation, thereby minimizing the loss of energy	Reductase	Catalase	Nitrogenase	Hydrogenase	Hydrogenase
6	A free-living anaerobic photosynthetic bacterium	*Anabaena azollae*	*Clostridium thermocellum*	*Rhodospirillum rubrum*	*Klebsiella pneumoniae*	*Rhodospirillum rubrum*
7	A free-living soil bacteria that is involved in nitrogen fixation	*Alcaligenes*	*Acetobacter*	*Pseudomonas*	*Azotobacter*	*Azotobacter*
8	Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biological nitrogen fixation	8	16	32	64	16
9	Apart from biological nitrogen fixation by microbes, _________ can fix atmospheric nitrogen	Cyclone	Thunder	Raining	Lightning	Lightning
10	Bacteria that forms root nodules in legume plants	*Rhizobium*	*Azotobacter*	*Azospirillum*	Cyanobacteria	*Rhizobium*
11	Biological nitrogen fixation was discovered by	Winogradsky	Beijerinck	Pasteur	Koch	Beijerinck
12	Chemicals produced by the Rhizobia called _________ that cause the colonized root hairs to curl	Pod factors	Nod factors	Sod factors	Mod factors	Nod factors
13	Example of associative nitrogen fixation	Legume-Rhizobium	Rice-Azospirillum	Higher plants-Mycorrhizae	Azolla-Anabaena	Rice-Azospirillum
14	*Frankia is a*	Bacteria	Actinomycete	Fungi	Algae	Actinomycete

15	Group of irregularly shaped bacteria in root nodules are called as	Bacteroids	Asteroids	Mesteroids	Histeroids	Bacteroids
16	In biological nitrogen fixation, ____ moles of ammonia are produced from one mole of nitrogen gas	2	4	6	8	2
17	In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as	Cyanocysts	Nitrocycts	Heterocysts	Homocysts	Heterocysts
18	In root nodules, ___________ bind and regulate the levels of oxygen in the nodule	Teghemoglobin	Peghemoglobin	Leghemoglobin	Hemoglobin	Leghemoglobin
19	Legume plants belongs to	*Solanaceae*	*Rosaceae*	*Astraceae*	*Fabaceae*	*Fabaceae*
20	Most abundant gas in atmosphere	Nitrogen	Oxygen	Carbon dioxide	Hydrogen	Nitrogen
21	Nitrogenase enzyme consists of	Iron protein	Molybdenum-iron protein	Iron protein and a molybdenum-iron protein	Hemoglobin	Iron protein and a molybdenum-iron protein
22	Rhizobia are attracted to ___________ released by the host legume's roots	Flavonoids	Enzymes	Toxins	Chemicals	Flavonoids
23	The enzyme nitrogenase is inhibited by	CO2	Sulfur	Hydrogen	Oxygen	Oxygen
24	*Which is not true about Anabaena and Nostoc*	Filamentous	Nitrogen fixing	Cyanobacteria	Symbiotic	Symbiotic
25	The majority of hydrogenases in prokaryotes are ________ containing enzymes	Nickel	Copper	Molybdenum	Sulfur	Nickel
26	The majority of hydrogenases in prokaryotes are ________ containing enzymes	Nickel	Copper	Molybdenum	Sulfur	Nickel
27	With associative nitrogen fixation, which one of the following genera is associated?	Azotobacter	Escherichia	Rhizobium	Anabena	Azotobacter
28	The conversion of nitrogen to ammonia or nitrogenous compounds is called as______	Nitrogen assimilation	Nitrogen fixation	Denitrification	Nitrification	Nitrogen fixation
29	Symbiotic nitrogen cyanobacteria are present in all except______	Anthoceros	Azolla	Cycas	Gnetum	Gentum

30	All the following are free living nitrogen fixers except________	Rhizobium	Azotobacter	Rhodospirillum	Clostridium	Rhizobium
31	Anabena is a nitrogen fixer present in the root pockets of_______	Marselia	Salvinia	Pistia	Azolla	Azolla
32	Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by _________	hydrogenase	nitrogenase	dinitrogenase	nitrate reductase	nitrogenase
33	Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere?	Bacteria	Algae	Nematodes	Moulds	Bacteria
34	A major plant macronutrient found in nucleic acids and proteins is _________	calcium	nitrogen	sulphur	iron	nitrogen
35	Organisms capable of converting nitrogen to nitrate are_______	yeast	bacteria	roundworms	moulds	bacteria
36	Conversion of nitrite to nitrate is carried out by _______	Nitrosomonas	Nitrosococcus	Nitrobacter	Clostridium	Nitrobacter
37	The nonsymbiotic bacteria which fix nitrogen live in the soil independently are	Azotobacter	Anabena	Rhizobium	Azolla	Azotobacter
38	Which of the following is not the biofertilisers producing bacteria?	Nostoc	Anabaena	Both (a) and (b)	Clostridium	Clostridium
39	Which of the following is capable of oxidizing sulfur to sulfates?	*Thiobacillus thiooxidans*	*Desulfotomaculum*	*Rhodospirillum*	*Rhodomicrobium*	Thiobacillus thiooxidans
40	Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrotrophic bacteria. The reasons may be due to	slow growth	medium growth	fast growth	no growth	slow growth
41	_______ play a key role in the transformatio nof rock in the transformation of rock to soil	Cyanobacterium	pectin decomposing bacteria	denitrifying bacteria		Cyanobacteria
42	Denitrification may be distinguished as	dissimilative and assimilative	assimilative	Partially dissimilative	Partially assimilative	Dissimilative and assimilative
43	All of the following are examples of negative symbiosis________	amensalism	competition	commensalism	parasitism	competition
44	The reservior for nitrogen is __________	the atmosphere	rocks	ammonia	nitrates	the atmosphere

45	Most soil protozoa are flagellates or amoebas, having their dominant mode of nitrogan as	Ingestion of bacteria	ingestion of mold	ingestion of fungi	ingestion virus	ingestion of bacteria
46		slow growth	medium growth	fast growth	Good growth	slow growth
47	Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrotrophic bacteria. The reasons may be due to					
48	The transformation of nitrates to gaseous nitrogen is accomplished by microorganisms in a series of biochemical reactions. The process is known as _____________	nitrification	denitrification	nitrogan fixation	ammonification	Denitrification
49	Nitrogen fixation refers to the direct conversion of atmospheric nitrogen gas into______	ammonia	glucose	ATP	Nitrate	ammonia
50	The diagnostic enzyme for denitrification is_________	nitrate reductase	nitrate oxidase	nitro oxidoreductase	reductase	nitrate reductase
51	An example of a symbiotic nitrogen fixer is________	Azotobacter	Beijerinckia	Clostridium	Rhizobium	Rhizobium
52	In the process of nitrogen fixation, which of the following microorganism is involved?	Non symbiotic microorganisms only	Symbiotic microorganisms only	Non symbiotic and symbiotic microorganisms only	Symbiosis	Non symbiotic and symbiotic microorganisms only
53	The physical structure of soil is improved by the accumulation of _______	mold mycelium	minerals	water	metals	Mold mycelium
54	________ play a key role in the transformation of rock to soil	Cyanobacteria	Pectin decomposing bacteria	Nitrifying bacteria	De-nitrifying bacteria	Cyanobacteria
55	The conversion of molecular nitrogen into ammonia is known as_______	nitrification	denitrification	nitrogen fixation	ammonification	nitrogen fixation
56	Some microorganisms have the ability to increase the nitrogen content of soils, are called as	Nitrogen fixation	denitrification	nitrification	ammonification	nitrogen fixation

57	Which of the following soil microorganism is involved in the reduction of sulfates to H2S	Thiobacillus thiooxidans	*Desulfotomaculum*	*Rhodospirillum*	*Rhodomicrobium*	*Desulfotomaculum*
58	Which of the following fungi on infecting crop roots can improve their uptake of phosphorus and other nutrients?	*Saccharomyces cerevisiae*	VA Mycorrhiza	*Candida torulopsis*	*Aspergillus niger*	VA Mycorrhiza
59	Syntrophism involves	exchange of nutrients between two species	exchange of nutrients among species	no exchange of nutrients between two species	no exchange of nutrients among species	exchange of nutrients between two species
60	Assimilative denitrification is done by _______	Plants	animals	virus	protozoans	plants
61	The diagnostic enzyme for nitrogen-fixing organisms is	nitrogenase	nitrate reductase	nitrate oxidase	dehrogenase	nitrogenase
62	The groups of bacteria which have the ability to fix nitrogen from air to soil are	Symbiotic	Antagonostic	Mutualistic	synergistics	Symbiotic
63	The nitrogenase consists of	dinitrogenase	reductase	hydrogenase	nitrogenase	dinitrogenase
64	The conversion of molecular nitrogen into ammonia is known as	nitrification	denitrification	nitrogen fixation	ammonification	nitrogen fixation
65	Some microorganisms have the ability to increase the nitrogen content of soils, are called as	nitrogen fixation	denitrification	nitrification	nitrogenase	nitrogen fixation
66	Which are the main source of biofertilisers?	Cyanobacteria	Bacillus	Streptococcus	Azolla	Cyanobacteria
67						
68	The physical structure of soil is improved by the accumulation of ________	Mold mycelium	minerals	water	yeast	mold mycelium
69	_______ play a key role in the transformatio nof rock in the transformation of rock to soil	Cyanobacterium	pectin decomposing bacteria	denitrifying bacteria		Cyanobacteria
70	Denitrification may be distinguished as	dissimilative and assimilative	assimilative	Partially dissimilative	Partially assimilative	Dissimilative and assimilative
71	All of the following are examples of	amensalism	competition	commensalism	parasitism	competition

	negative symbiosis________					
72	The reservior for nitrogen is __________	the atmosphere	rocks	ammonia	nitrates	the atmosphere
73	When a host associates with other organism for either benefit or completion of their life cycle is known as ________	Cooperation	Mutualism	Symbiosis	Synergism	Symbiosis
74	An obligatory relationship between host and its symbionts is known as __________	Cooperation	Mutualism	Symbiosis	Synergism	Mutualism
75	Which onr of the following presence and contributions of microorganisms through their activities to the places where they are found	Environmental microbiology	Microbial ecology	Microbial physiology	Microbial taxonomy	Microbial ecology
76	The relationship in which one symbionts benefits while the other is neither harmed nor help is known as	Cooperation	Synergism	Syntrophism	Commensalism	Commensalism
77	Which of the following describes that one organism has deverse effect on another organism	amensalism	Commensalism	Mutulism	Cooperation	Amensalism
78	Which of the following describes an example of ammensalism in soil ecosystem	Production of antibiotics	Production of secondary metabolite	Production of geosmine	Production of primary metabolites	Production of antibiotics
79	The aphids is an excellent example of which one of the following relationship	Mutualism	Cooperation	Symbiosis	Synergism	Mutualism

UNIT – V (Biofertilizers)

Biofertilizer - Application of biofertilizers and biomanures – A combination of biofertilizer and manure applications with reference to soil, seed and leaf sprays. Laboratory and field application; Cost-benefit analysis of biofertilizer and biomanure production. Biopesticides and its application

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

Very often microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

Rhizobium: *Rhizobium* is a soil habitat bacterium, which can able to colonize the legume roots and fixes the atmospheric nitrogen symbiotically. The morphology and physiology of *Rhizobium* will vary from free-living condition to the bacteroid of nodules. They are the most efficient biofertilizer as per the quantity of nitrogen fixed concerned. They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group. *Rhizobium* inoculant was first made in USA and commercialized by private enterprise in 1930s and the strange situation at that time has been chronicled by Fred (1932).

Initially, due to absence of efficient bradyrhizobial strains in soil, soybean inoculation at that time resulted in bumper crops but incessant inoculation during the last four decades by US farmers has resulted in the build up of a plethora of inefficient strains in soil whose replacement by efficient strains of bradyrhizobia has become an insurmountable problem.

***Rhizobium*-characters**: This belongs to bacterial group and the classical example is symbiotic nitrogen fixation. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is reality utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of *Rhizobium*. The percentage of nodules occupied, nodules dry weight, plant dry weight and the grain yield per plant the multistrain inoculant was highly promising Table-2 shows the N fixation rates.

Seed Treatment: Seed treatment is a most common method adopted for all types of inoculants. The seed treatment is effective and economic. For small quantity of seeds (up to 5 kgs quantity) the coating can done in a plastic bag. For this purpose, a plastic bag having size (21" x 10") or big size can be used. The bag should be filled with 2 kg or more of seeds. The bag should be closed in such a way to trap the airs as much as possible. The bag should be squeezed for 2 minutes or more until all the seed are uniformly wetted. Then bag is opened, inflated again and shaked gently. Stop shaking after each seeds gets a uniform layer of culture coating. The bag is opened and the seed is dried under the shade for 20-30 minutes. For large amount of seeds coating can be done in a bucket and inoculant can be mixed directly with hand. Seed Treatment with *Rhizobium*, *Azotobacter, Azospirillum*, along with PSM can be done.

The seed treatment can be done with any of two or more bacteria. There is no side (antagonistic) effect. The important things that have to be kept in mind are that the seeds must be coated first with *Rhizobium, Azotobacter* or *Azospirillum*. When each seed get a layer of above bacteria then PSM inoculant has to be coated as outer layer. This method will provide maximum number of each bacterium required for better results. Treatments of seed with any two bacteria will not provide maximum number of bacteria on individual seed.

Essential equipments

Autoclave: It is an apparatus in which materials are sterilized by air free saturated steam (under pressure) at a temperature above $100^{O}C$. If the steam pressure inside the autoclave is increased to 15 psi, the temperature will rise to 121°C. This is sufficient to destroy all vegetative cells. Normally all growth medium are sterilized in the autoclave.

Laminar air flow chamber: Laminar air flow chamber provides a uniform flow of filtered air. This continuous flow of air will prevent settling of particles in the work area.Air borne contamination is avoided in this chamber. Culture transfers and inoculation can be done here.

Rotary shaker: It is used for agitating culture flasks by circular motion under variable speed control. Shaking provides aeration for growth of cultures. Shakers holding upto 20-50 flasks are generally used. The capacity of the shaker may be increased if it is a double- decker type.

Hot air oven: Hot air oven is meant for sterilizing all glassware materials. Dry heat is used in this apparatus to sterilize the materials. Normally 180OC is used for two hours for sterilizing glasswares.

pH meter: An instrument for measuring pH of the solution using a 0-14 scale in which seven represents neutral points, less than seven is acidity (excess of H‘ over OH-) and more than seven is alkality (excess of OH- over H‘) useful in adjusting the pH of the growth medium.

Refrigerator: This equipment is used preserving all mother cultures used for biofertilizer production. The mother culture is periodically sub-cultured and stored in the refrigerator for long- term usage.

Fermentor: A fermentor is the equipment, which provides the proper environment for the growth of a desired organism.It is generally a large vessel in which, the organism may be kept at the required temperature, pH , dissolved oxygen concentration and substrate concentration. Different models of fermentors are available depending upon the necessity.A simple version model contains steam generator, sterilization process devices and agitator.A sophisticated fermentor contains pH regulator, oxygen level regulator, anti-foam device, temperature controller, etc.

Culturing of Microorganisms: Although many bacteria can be used beneficially as a biofertilizer the technique of mass production is standardizedfor *Rhizobium*, *Azospirillum*, *Azotobacter* and phosphobacteria. The media used for mass culturing ;

Rhizobium : Yeast extract mannitol broth.

Growth on Congo red yeast extract mannitol agar medium: Add 10 ml of Congo red stock solution (dissolve 250 mg of Congo red in 100ml water) to 1 liter after adjusting the PH to 6.8 and before adding agar. *Rhizobium* forms white, translucent, glistening, elevated and comparatively small colonies on this medium. Moreover, *Rhizobium* colonies do not take up the colour of congo red dye added in the medium. Those colonies which readily take up the congo red stain are not rhizobia but presumably *Agrobacterium*, a soil bacterium closely related to *Rhizobium.*

Inoculum preparation

- Prepare appropriate media for specific to the bacterial inoculant in 250 ml, 500 ml, 3 litre and 5 litre conical flasks and sterilize.
- The media in 250 ml flask is inoculated with efficient bacterial strain under aseptic condition
- Keep the flask under room temperature in rotary shaker (200 rpm) for 5- 7 days.
- Observe the flask for growth of the culture and estimate the population, which serves as the starter culture.
- Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 litre and 5 litre) containing the media, after obtaining growth in each flask.
- The above media is prepared in large quantities in fermentor, sterilized well, cooled and kept it ready.
- The media in the fermentor is inoculated with the log phase culture grown in 5 litre flask. Usually 1 -2 % inoculum is sufficient, however inoculation is done up to 5% depending on the growth of the culture in the larger flasks.
- The cells are grown in fermentor by providing aeration (passing sterile air through compressor and sterilizing agents like glass wool, cotton wool, acid etc.) and given continuous stirring.
- The broth is checked for the population of inoculated organism and contamination if any at the growth period.
- The cells are harvested with the population load of 109 cells ml-1 after incubation period.
- There should not be any fungal or any other bacterial contamination at 10-6 dilution level

- It is not advisable to store the broth after fermentation for periods longer than 24 hours. Even at 4o C number of viable cells begins to decrease.

Processing of carrier material

The use of ideal carrier material is necessary in the production of good quality biofertilizer. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. The neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production The following points are to be considered in the selection of ideal carrier material.

- Cheaper in cost
- Should be locally available
- High organic matter content
- No toxic chemicals
- Water holding capacity of more than 50%
- Easy to process, friability and vulnerability.
- The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio) , since the peat soil / lignite are acidic in nature (pH of 4 - 5)
- The neutralized carrier material is sterilized in an autoclave to eliminate the contaminants.

Mixing the carrier and the broth culture and packing: Inoculant packets are prepared by mixing the broth culture obtained from fermentor with sterile carrier material as described below:

Preparation of Inoculants packet

- The neutralized, sterilized carrier material is spread in a clean, dry, sterile metallic or plastic tray.
- The bacterial culture drawn from the fermentor is added to the sterilized carrier and mixed well by manual (by wearing sterile gloves) or by mechanical mixer. The culture suspension is to be added to a level of 40 – 50% water holding capacity depending upon the population.
- The inoculant packet of 200 g quantities in polythene bags, sealed with electric sealer and allowed for curing for 2 -3 days at room temperature (curing can be done by spreading the inoculant on a clean floor/polythene sheet/ by keeping in open shallow tubs/ trays

with polythene covering for 2 -3 days at room temperature before packaging).

Specification of the polythene bags

- The polythene bags should be of low density grade.
- The thickness of the bag should be around 50 – 75 micron.
- Each packet should be marked with the name of the manufacturer, name of the product, strain number, the crop to which recommended, method of inoculation, date of manufacture, batch number, date of expiry, price, full address of the manufacturer and storage instructions etc.,

Storage of biofertilizerpacket

- The packet should be stored in a cool place away from the heat or direct sunlight.
- The packets may be stored at room temperature or in cold storage conditions in lots in plastic crates or polythene / gunny bags.
- The population of inoculant in the carrier inoculant packet may be determined at 15 days interval. There should be more than 109 cells / g of inoculant at the time of preparation and107 cells/ g on dry weight basis before expiry date.

Azospirillum: *Azospirillum lipoferum* and *A. brasilense* (Spirillum lipoferum in earlier literature) are primary inhabitants of soil, the rhizosphere and intercellular spaces of root cortex of graminaceous plants. They perform the associative symbiotic relation with the graminaceous plants.

The bacteria of Genus Azospirillum are N2 fixing organisms isolated from the root and above ground parts of a variety of crop plants. They are Gram negative, Vibrio or Spirillum having abundant accumulation of polybetahydroxybutyrate (70 %) in cytoplasm.

Five species of Azospirillum have been described to date A. brasilense, A.lipoferum, A.amazonense, A.halopraeferens and A.irakense. The organism proliferates under both anaerobic and aerobic conditions but it is preferentially micro-aerophilic in the presence or absence of combined nitrogen in the medium.

Apart from nitrogen fixation, growth promoting substance production (IAA), disease resistance and drought tolerance are some of the additional benefits due to Azospirillum inoculation.

Preparation of Mother or Starter Cultures: Starter cultures of selected strains are obtained after ascertaining their performance in green house and at field levels. The pure culture of efficient strain of nitrogen fixing organism is grown on respective agar medium on slant and maintained in the laboratory. A loopful of inoculum from the slant is transferred in a 250 ml capacity conical flask containing liquid medium. keep the conical flak on rotary shaker for 3-7 days depending whether they are fast growing or slow growing. The content of these flasks usually attain a load of 10 5- 10 6 cells per ml called mother culture or starter culture. This mother cultures are further multiplied in larger flasks.

Preparation of Broth Cultures

Prepare liquid medium for respective organisms. Distribute equal quantity in big conical flasks (1000 ml). Sterilize it in autoclave for half an hour at 15 lbs pressure. After sterilization each flask containing suitable broth is inoculated with the mother culture in 1:5 proportions aseptically. Keep the flaks on rotary shaker for 96-120 hours until the viable count per ml reaches to 10 9 cells. The broths become more thick in consistency. This broth culture with population of 10 9 cells or ml should not be stored more than 24 hours or stored at 4 0C temperature.

Preparation of Carrier

The carrier should have following characters

a) It should have high organic matter above 60%.

b) Low soluble salts less than 1%.

c) High moisture holding capacity 150 to 200% by weight.

d) Provide a nutritive medium for growth of bacteria and prolong their survival in culture as well as on inoculated seed.

Lignite or peat is used as carrier in the preparation of Biofertilizers. The carriers are crushed and powdered to 200 to 300 mesh. Peat or Lignite powder is neutralized by addition of 1% calcium carbonate ($CaCO_3$) and sterilized at 15 lbs pressure for 3-4 hours in autoclave.

Preparation of Inoculate

The sterilized and neutralized lignite or peat is mixed with high count broth culture in galvanised trays. About 1 part by weight of broth is required to 2 part of dry carrier. Final moisture content varies from 40 to 50% depending upon quality of carriers.

Curing or Maturation: After mixing the broth cultures and lignite or peat powder in 1:2 proportion in the galvanised trays then it is kept for curing at room temp (28 0C) for 5 to 10 days. After curing it is sieved to disperse the concentrated pockets of growth and to break the lumps.

Filling and Packing: After curing, sieved powder is filled in polythene bagas of 0.5 mm thickness leaving 2/3 space open for aeration of the bacteria. Bag is weighted for desired quantity. Then the bag is packed by sealing. The polythene bag used for filling microbial inoculant should be printed with following information.

a) Name of Inoculants

b) Direction for use

c) Name of crops

d) Date of Manufacture.

e) Date of expiry.

Quality Checking

Check viable count in the carrier based inoculants by dilution plate method at the time of manufactures. The viable cells count in the carrier based inoculants should be maintained as per ISI specifications.

Storage

The inoculants shall be stored by the manufacture in a cool place away from direct heat preferably at a temp of 15 ^{0}C and not exceeding 30 ^{0}C +- 2 ^{0}C for six months. For long survival of microorganisms the bag are stored in cold storage at 4 ^{0}C temp.

Azotabacter: The several species of Azotobacter, A. chroococcum happens to be the dominant inhabitant in arable soils capable of fixing N2 (2-15 mg N2 fixed /g of carbon source) in culture media.

The bacterium produces abundant slime which helps in soil aggregation. The numbers of A. chroococcum in Indian soils rarely exceeds 105/g soil due to lack of organic matter and the presence of antagonistic microorganisms in soil.

It is the important and well known free living nitrogen fixing aerobic bacterium. It is used as a Bio-Fertilizer for all non leguminous plants especially rice, cotton, vegetables etc. Azotobacter cells are not present on the rhizosplane but are abundant in the rhizosphere region. The lack of organic matter in the soil is a limiting factor for the proliferation of Azotobaceter in the soil.

Field experiments were conducted in 1992, 1993 and 1994 during the pre-kharif wet seasons to find out the influence on rice grain yield by the combined use of N- fixing organisms and inorganic nitrogen fertilizer which recorded increase in was yield.

The pigmentation that is produced by *Azotobacter* in aged culture is melanin which is due to oxidation of tyrosine by tyrosinase an enzyme which has copper. The colour can be noted in liquid forms. Some of the pigmentation are described below-

A. chroococcum: Produces brown-black pigmentation in liquid inoculum.

A. beijerinchii: Produces yellow- light brown pigementation in liquid inoculum

A. vinelandii: Produces green fluorescent pigmentation in liquid inoculum.

A. paspali: Produces green fluorescent pigmentation in liquid inoculum.

A. macrocytogenes: Produces, pink pigmentation in liquid inoculum.

A. insignis: Produces less, gum less, grayish-blue pigmentation in liquid inoculum.

A. agilies: Produces green-fluorescent pigmentation in liquid inoculum.

The performance of *Azotobacter* liquid inoculant was comparatively better than all the treatments in 10 % MS medium followed *Azospirillum*.The performance of *Azotobacter* liquid inoculant was comparatively better than all the treatments followed by *Azosopirillum* for the growth of the polybag sugarcane seedlings.

Preparation of Mother or Starter Cultures: Starter cultures of selected strains are obtained after ascertaining their performance in green house and at field levels. The pure culture of efficient strain of nitrogen fixing organism is grown on respective agar medium on slant and maintained in the laboratory. A loopful of inoculum from the slant is transferred in a 250 ml capacity conical flask containing liquid medium. keep the conical flak on rotary shaker for 3-7 days depending whether they are fast growing or slow growing. The content of these flasks usually attain a load of 10 5- 10 6 cells per ml called mother culture or starter culture. This mother cultures are further multiplied in larger flasks.

Preparation of Broth Cultures: Prepare liquid medium for respective organisms. Distribute equal quantity in big conical flasks (1000 ml). Sterilize it in autoclave for half an hour at 15 lbs pressure. After sterilization each flask containing suitable broth is inoculated with the mother culture in 1:5 proportions aseptically. Keep the flaks on rotary shaker for 96-120 hours until the viable count per ml reaches to 10^9 cells. The broths become more thick in consistency. This broth culture with population of 10 9 cells or ml should not be stored more than 24 hours or stored at 4 ^{0}C temperature.

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Preparation of Inoculate : The sterilized and neutralized lignite or peat is mixed with high count broth culture in galvanised trays. About 1 part by weight of broth is required to 2 part of dry carrier. Final moisture content varies from 40 to 50% depending upon quality of carriers.

After mixing the broth cultures and lignite or peat powder in 1:2 proportion in the galvanised trays then it is kept for curing at room temp (28 ^{0}C) for 5 to 10 days. After curing it is sieved to disperse the concentrated pockets of growth and to break the lumps.

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Quality Checking: Check viable count in the carrier based inoculants by dilution plate method at the time of manufactures. The viable cells count in the carrier based inoculants should be maintained as per ISI specifications.

Storage: The inoculants shall be stored by the manufacture in a cool place away from direct heat preferably at a temp of 15 0C and not exceeding 30 ^{0} C +- 2 ^{0} C for six months. For long survival of microorganisms the bag are stored in cold storage at 4 ^{0} C temp.

Gluconaccetobacter: Sugar Plus is a biofertilizer for sugarcane. Sugar Plus's active ingredient is a very significant symbioticnitrogen-fixing bacterium for sugarcane.It was first discovered in high yielding varieties of sugarcane grown in Brazil. As per international research findings up to 40% biological nitrogen fixation in sugarcane is due to these bacteria.

It addition to its nitrogen fixation it also secrets useful growth promoting hormones such as Insole Acetic Acid (IAA).

Azorhizobium: It is a stem nodule forming bacteria and fixes nitrogen symbionts of the stem nodule also produce large amount of IAA that promotes plant growth.

Preparation of Mother or Starter Cultures: Starter cultures of selected strains are obtained after ascertaining their performance in green house and at field levels. The pure culture of efficient strain of nitrogen fixing organism is grown on respective agar medium on slant and maintained in the laboratory. A loopful of inoculum from the slant is transferred in a 250 ml capacity conical flask containing liquid medium. keep the conical flak on rotary shaker for 3-7 days depending whether they are fast growing or slow growing. The content of these flasks usually attain a load of 10^{5}- 10^{6} cells per ml called mother culture or starter culture. This mother cultures are further multiplied in larger flasks.

Preparation of Broth Cultures: Prepare liquid medium for respective organisms. Distribute equal quantity in big conical flasks (1000 ml). Sterilize it in autoclave for half an hour at 15 lbs pressure. After sterilization each flask containing suitable broth is inoculated with the mother culture in 1:5 proportions aseptically. Keep the flaks on rotary shaker for 96-120 hours until the viable count per ml reaches to 10^{9} cells. The broths become more thick in consistency. This broth culture with population of 10 9 cells or ml should not be stored more than 24 hours or stored at $4\ ^{0}$C temperature.

Preparation of Inoculate: The sterilized and neutralized lignite or peat is mixed with high count broth culture in galvanised trays. About 1 part by weight of broth is required to 2 part of dry carrier. Final moisture content varies from 40 to 50% depending upon quality of carriers.

Curing or Maturation: After mixing the broth cultures and lignite or peat powder in 1:2 proportion in the galvanised trays then it is kept for curing at room temp (28 ^{0}C) for 5 to 10 days. After curing it is sieved to disperse the concentrated pockets of growth and to break the lumps.

Phosphobacteria: Inoculated through seed, seedling root dip and soil application methods as in the case of *Azospirillum*. Combined application of bacterial biofertilizers. Phosphobacteria can be mixed with *Azospirillum* and *Rhizobium*. The inoculants should be mixed in equal quantities and applied as mentioned above. The recommended dosage of *Azospirillum* is adopted for phosphobacteria inoculation; for combined inoculation, both biofertilizers as per recommendations are to be mixed uniformly before using.

Blue Green Algae (BGA): Blue green algal inoculation with composite cultures was found to be more effective than single culture inoculation. A technology for mass scale production of composite culture of blue green algae under rice field condition was developed at TNAU and the soil based BGA inoculum could survive for more than 2 years.

At many sites where algal inoculation was used for three to four consecutive cropping seasons, the inoculated algae establish well and the effect persisted over subsequent rice crop. Technologies for utilizing nitrogen fixing organisms in low land rice were the beneficial role of blue green algal inoculation in rice soils of Tamil Nadu.

The blue green algal inoculum may be produced by several methods viz., in tubs, galvanized trays, small pits and also in field conditions. However the large-scale production is advisable under field condition which is easily adopted by farmers.

Multiplication in trays

- Big metallic trays (6’x 3’x 6”lbh) can be used for small scale production
- Take 10 kg of paddy field soil, dry powder well and spread

- Fill water to a height of 3”
- Add 250 g of dried algal flakes (soil based) as inoculum
- Add 150 g of super phosphate and 30 g of lime and mix well with the soil
- Sprinkle 25 g carbofuran to control the insects
- Maintain water level in trays
- After 10 to 15 days, the blooms of BGA will start floating on the water sources
- At this stage stop watering and drain. Let the soil to dry completely
- Collect the dry soil based inoculum as flakes
- Store in a dry place. By this method 5 to 7 kg of soil based inoculum can be obtained.

Multiplication under field condition

Materials

- Rice field
- Super phosphate
- Carbofuran
- Composite BGA starter culture

Procedure

- Select an area of 40 m2 (20m x 2m) near a water source which is directly exposed to sunlight.
- Make a bund all around the plot to a height of 15 cm and give it a coating with mud to prevent loss of water due to percolation.
- Plot is well prepared and levelled uniformly and water is allowed to a depth of 5-7.5 cm and left to settle for 12 hrs.
- Apply 2 kg of super phosphate and 200 g lime to each plot uniformly over the area.
- The soil based composite starter culture of BGA containing 8-10 species @ 5 kg / plot is powdered well and broadcasted.
- Carbofuran @ 200 g is also applied to control soil insects occurring in BGA.
- Water is let in at periodic intervals so that the height of water level is always maintained at 5 cm.

- After 15 days of inoculation, the plots are allowed to dry up in the sun and the algal flakes are collected and stored.

Observations: The floating algal flasks are green or blue green in colour. From each harvest, 30 to 40 kg of dry algal flakes are obtained from the plot**.**

Plant Growth Promoting Rhizobacteria (PGPR): Plant growth promoting rhizobacteria (PGPR) have been studied for long. It has been suggested in the last few years that endophytic N2-fixing bacteria may be more important than rhizospheric bacteria in promoting plant growth because they escape competition with rhizosphere microorganisms and achieve close contact with the plant tissues. The well known genera of PGPR are Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, and Pseudomonas, but some of these genera include endophytic species as well. The best-characterized endophytic bacteria include Azoarcus spp, Gluconacetobacter diazotrophicus, and Herbaspirillum seropedicae.

Associative nitrogen fixation: Many PGPR and endophytic bacteria can grow diazotrophically, and either be symbiotic or non-symbiotic. Young (1992) has reviewed the phylogenetic classification of nitrogen-fixing organisms, but in the last few years many novel N2-fixing species belonging to different genera have been described.

A genomic-based survey for nitrogen-fixing genes indicates that approximately 5% of prokaryotes could carry nitrogen fixation-like genes (Raymond et al., 2004). Nitrogen-fixation genes are plasmid borne in some species, but most prokaryotes have chromosomal nif genes. Hence, it would not be so simple for them to loose nif genes. Hitherto, the presence of the novel superoxide-dependent nitrogen fixing system detected in Streptomyces thermoautotrophicus has not been reported in other bacteria, raising the possibility that several known or unknown PGPR could have it.

Considering carbon supply is one of the limiting factors for associative nitrogen fixation in non-legumes it would be desirable to look for cultivars that excrete photosyntates in adequate amounts in order to find effective nitrogen fixation in rhizospheric associations. Gyaneshwar found significant uptake of 15N2 by one rice variety that exuded a great quantity of carbon compounds.

Azolla: *Azolla* is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga Anabaena azollae. *Azolla* fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. Rice growing areas in South East Asia and other third World countries have recently been evincing increased interest in the use of the symbiotic N2 fixing water fern *Azolla* either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. *Azolla* is used as biofertilizer for wetland rice and it is known to contribute 40-60 kg N ha-1 per rice crop. The agronomic potential of *Azolla* is quite significant particularly for rice crop and it is widely used as biofertilizer for increasing rice yields. Rice crop response studies with *Azolla* biofertilizer in the People's Republic in China and in Vietnam have provided good evidence that *Azolla* incorporation into the soil as a green manure crop is one of the most effective ways of providing nitrogen source for rice.

The utilization of *Azolla* as dual crop with wetland rice is gaining importance in Philippines, Thailand, Srilanka and India. The important factor in using *Azolla* as a biofertilizer for rice crop is its quick decomposition in soil and efficient availability of its nitrogen to rice. In tropical rice soils the applied *Azolla* mineralizes rapidly and its nitrogen is available to the rice crop in very short period. The common species of *Azolla* are *A. microphylla, A. filiculoides, A. pinnata, A. caroliniana, A. nilotica, A. rubra and A. mexicana.*

Mass multiplication of *Azolla* under field conditions

A simple *Azolla* nursery method for large scale multiplication of *Azolla* in the field has been evolved for easy adoption by the farmers.

Materials

- One cent (40 sq.m) area plot
- Cattle dung
- Super phosphate
- Furadan
- Fresh Azolla inoculum

Procedure: Mark the field into one cent plots (20 x 2m) by providing suitable bunds and irrigation channels. Maintain water level to a height of 10 cm. Mix 10 kg of cattle dung in 20 litres of water and sprinkle in the field. Apply 100 g super phosphate as basal dose. Inoculate fresh Azolla biomass @ 8 kg to each pot. Apply super phosphate @ 100 g as top dressing fertilizer on 4th and 8th day after Azolla inoculation. Apply carbofuran (furadan) granules @ 100 g/plot on 7th day after Azolla inoculation. Maintain the water level at 10 cm height throughout the growth period of two or three weeks.

Method of inoculation of Azolla to rice crop: The Azolla biofertilizer may be applied in two ways for the wetland paddy. In the first method, fresh Azolla biomass is inoculated in the paddy field before transplanting and incorporated as green manure. This method requires huge quantity of fresh Azolla. In the other method, Azolla may be inoculated after transplanting rice and grown as dual culture with rice and incorporated subsequently.

Azolla biomass incorporation as green manure for rice crop: Collect the fresh Azolla biomass from the Azolla nursery plot. Prepare the wetland well and maintain water just enough for easy incorporation. Apply fresh Azolla biomass (15 t ha-1) to the main field and incorporate the Azolla by using implements or tractor.

***Azolla* inoculation as dual crop for rice**: Select a transplanted rice field. Collect fresh *Azolla* inoculum from *Azolla* nursery. Broadcast the fresh *Azolla* in the transplanted rice field on 7th day after planting (500 kg / ha). Maintain water level at 5-7.5cm. A second bloom of *Azolla* will develop 8 weeks after transplanting which may be incorporated again. By the two incorporations, 20-25 tonnes of *Azolla* can be incorporated in one hectare rice field.

Production and quality control of biofertilizers: Though the biofertilizer technology is a low cost, ecofriendly technology, several constraints limit the application or implementation of the technology the constraints may be environmental, technological, infrastructural, financial, human resources, unawareness, quality, marketing, etc. The different constraints in one way or other affecting the technique at production, or marketing or usage.

Technological constraints

- Use of improper, less efficient strains for production.
- Lack of qualified technical personnel in production units.
- Unavailability of good quality carrier material or use of different carrier materials by different producers without knowing the quality of the materials.
- Production of poor quality inoculants without understanding the basic microbiological techniques
- Short shelf life of inoculants.
- Infrastructural constraints
- Non-availability of suitable facilities for production
- Lack of essential equipments, power supply, etc.
- Space availability for laboratory, production, storage, etc. •
- Lack of facility for cold storage of inoculant packets

Financial constraints

- Non-availability of sufficient funds and problems in getting bank loans
- Less return by sale of products in smaller production units.

Environmental constraints

- Seasonal demand for biofertilizers.
- Simultaneous cropping operations and short span of sowing/planting in a particular

locality.

- Soil characteristics like salinity, acidity, drought, water logging, etc.

Human resources and quality constraints

- Lack of technically qualified staff in the production units.
- Lack of suitable training on the production techniques.
- Ignorance on the quality of the product by the manufacturer
- Non-availability of quality specifications and quick quality control methods
- No regulation or act on the quality of the products

Awareness on the technology

- Unawareness on the benefits of the technology
- Problem in the adoption of the technology by the farmers due to different methods of inoculation.
- No visual difference in the crop growth immediately as that of inorganic fertilizers.
- Unawareness on the damages caused on the ecosystem by continuous application of inorganic fertilizer.

Marketing constraints

- Non availability of right inoculant at the right place in right time.
- Lack of retain outlets or the market network for the producers.

Field applications and crop response

Application of Biofertilizers

1. Seed treatment or seed inoculation

2. Seedling root dip

3. Main field application

Seed treatment: One packet of the inoculant is mixed with 200 ml of rice kanji to make a slurry. The seeds required for an acre are mixed in the slurry so as to have a uniform coating of the inoculant over the seeds and then shade dried for 30 minutes. The shade dried seeds should be sown within 24 hours. One packet of the inoculant (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip: This method is used for transplanted crops. Two packets of the inoculant is mixed in 40 litres of water. The root portion of the seedlings required for an acre is dipped in the mixture for 5 to 10 minutes and then transplanted.

Main field application: Four packets of the inoculant is mixed with 20 kgs of dried and powdered farm yard manure and then broadcasted in one acre of main field just before transplanting.

Biopesticide: Biopesticides, a contraction of 'biological pesticides', include several types of pest management intervention: through predatory, parasitic, or chemical relationships. The term has been associated historically with biological control - and by implication - the manipulation of living organisms.

Bacillus thuringiensis: *Bacillus thuringiensis* (or Bt) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well on leaf surfaces, aquatic environments, animal feces, insect rich environments, flour mills and grain storage facilities.

During sporulation, many Bt strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have Insecticide action. This has led to their use as insecticides, and more recently to genetically modified crops using Bt genes. Many crystal-producing Bt strains, though, do not have insecticidal properties.

Use of spores and proteins in pest control: Spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been used to control insect pests since the 1920s and are often applied as liquid sprays They are now used as specific insecticides under trade names such as DiPel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects and are used in Organic farming however the manuals for these products do contain many environmental and human health warnings, and a 2012 European regulatory peer review of 5 approved strains found that while there is data to support some claims of low toxicity to humans and the environment, there is insufficient data to justify many of these claims.

Bacillus thuringiensis serovar israelensis, a strain of *B. thuringiensis* is widely used as a larvicide against mosquito larvae, where it is also considered an environmentally friendly method of mosquito control.

As, for example, insects develop resistance to Bt,or there is desire to force mutations to modify organism characteristics or to use homologous recombinant genetic engineering to improve crystal size and increase pesticidal activity or broaden the host range of Bt and obtain more effective formulations, etc., new strains of Bt are developed and introduced over time. Each new strain is given a unique number and registered with the U.S. EPA and allowances may be given for genetic modification depending on "its parental strains, the proposed pesticide use pattern, and the manner and extent to which the organism has been genetically modified". Formulations of Bt that are approved for organic farming in the US are listed at the website of the Organic Materials Review Institute (OMRI) and several university extension websites offer advice on how to use Bt spore or protein preparations in organic farming.

Insect Viruses : Insect viruses are attractive as biological control agents and could be a feasible alternative to chemical insecticides in the management of insect infestations. This review describes recent advances in the development of wild-type and genetically modified viruses as insecticides. A new strategy of application of insect viruses in China is reviewed. Also, the assessment of biosafety of genetically modified Helicoverpa armigera Nucleopolyhedovirus (HearNPV) is emphasized as a case-study.

Insect-specific viruses can be highly effective natural controls of several caterpillar pests. Different strains of naturally occurring nuclear polyhedrosis virus (NPV) and granulosis virus are present at low levels in many insect populations. Epizootics can occasionally devastate populations of some pests, especially when insect numbers are high.

Insect viruses need to be eaten by an insect to cause infection but may also spread from insect to insect during mating or egg laying. In some cases, for example while searching for suitable hosts for egg laying, beneficial insects such as parasitoids may physically spread a virus through the pest population.

No threat to humans or wildlife is posed by insect viruses. Virus diseases of caterpillar pests may cause indirect mortality of some beneficial larval parasitoids if the host insects die before the parasitoids have completed development. Predators and adult parasitoids are not directly affected. Viruses can overwinter in the environment or in overwintering insects to re-establish infection in subsequent seasons.

The successful commercialization of insect-pathogenic viruses has been limited. Thus far, NPV strains have only been mass produced in living insects, a costly procedure. Viral insecticide development is further hindered by the fact that the viruses are specific to one species or genus, ensuring a relatively small market.

Entemopathogenic fungi: These fungi usually attach to the external body surface of insects in the form of microscopic spores (usually asexual, mitosporic spores also called conidia). Under permissive conditions of temperature and (usually high) moisture, these spores germinate, grow as hyphae and colonize the insect's cuticle; eventually they bore through it and reach the insects' body cavity (hemocoel). Then, the fungal cells proliferate in the host body cavity, usually as walled hyphae or in the form of wall-less protoplasts (depending on the fungus involved). After some time the insect is usually killed (sometimes by fungal toxins) and new propagules (spores) are formed in/on the insect if environmental conditions are again permissive; usually high humidity is required for sporulation.

Groups: The entomopathogenic fungi include taxa from several of the main fungal groups and do not form a monophyletic group. Many common and/or important entomopathogenic fungi are in the order Hypocreales of the Ascomycota: the asexual (anamorph) phases Beauveria, Metarhizium, Nomuraea, Paecilomyces = Isaria, Hirsutella and the sexual (teleomorph) state Cordyceps; others (Entomophthora, Zoophthora, Pandora, Entomophaga) belong in the order Entomophthorales of the Zygomycota.

Pest control: Since they are considered natural mortality agents and environmentally safe, there is worldwide interest in the use and manipulation of entomopathogenic fungi for biological control of insects and other arthropod pests. In particular, the asexual phases of Ascomycota (Beauveria spp., Lecanicillium spp., Metarhizium spp., Paecilomyces spp. and others) are under intense scrutiny due to the traits favouring their use as biological insecticides.

Production: Most entomopathogenic fungi can be grown on artificial media. However, some require extremely complex media; others, like *Beauveria bassiana* and exploitable species in the genus *Metarhizium*, can be grown on starch-rich substrates like cereal grains (rice, wheat).

Virulence: The Entomophthorales are often reported as causing high levels of mortality (epizootics) in nature. These fungi are highly virulent. The anamorphic Ascomycota (Metarhizium, Beauveria etc.) are reported as causing epizootics less frequently in nature. Also important are their properties regarding specificity (host range), storage, formulation, and application.

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. What is biofertilizers?
2. Write about the benefit of biomanures?
3. Comment on biopesticides
4. Give a short notes about rhizobium
5. Comment on algae as a biofertilizer

PART-C (8 MARKS)

1. Describe the mass production of biofertilizers
2. Write a details notes about the application of biomanures
3. Explain the role of biopesticides and their applications
4. Write about the field application methods of microbial treatments to enhance the crop plants
5. Describe the importance of biofertilizers

S.No	Unit V	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	______ is organic matter, mostly derived from animal waste/feces	Biomanure	Fertilizer	Potash	NPK	Biomanure
2	______ is the used for seed treatment of groundnut	*Azospirillum*	*Azotobacter*	*Rhizobium*	*Nostoc*	*Rhizobium*
3	_______ are best phosphate mobilizers	*Mycorrhizae*	*Bacillus*	*Citrobacter*	*Candida*	*Mycorrhizae*
4	_______ is a biocontrol agent	*Bacillus polymyxa*	*Azospirillum*	*Trichoderma viridae*	*Aspergillus flavus*	*Trichoderma viridae*
5	________ are rich in beneficial microorganisms that enrich the nutrient quality of soil	Biofertilizers	Humus	NPK	Vermicompost	Biofertilizers
6	________ is a best biofertilizer used in paddy fields	*Bradyrhizobium*	*Azospirillum*	*Azolla*	*Frankia*	*Azospirillum*
7	________ is a form of agriculture that relies on techniques such as crop rotation, green manure, compost, and biological pest control.	Terrestial farming	Hill farming	Inorganic farming	Organic farming	Organic farming
8	________ is phosphate solubilizing bacteria	*Bacillus megaterium*	*Bacillus anthrax*	*Bacillus cereus*	*Bacillus phosphatae*	*Bacillus megaterium*
9	________ is the biological oxidation of ammonia	Oxidation	Nitrification	Denitrification	Reduction	Nitrification
10	_________ can be used with crops like wheat, maize,mustard, cotton, potato and other vegetable crops	*Anabaena*	*Azotobacter*	*Rhizobium*	*Mycorrhizae*	*Azotobacter*
11	_________ is a plant growth promoting bacteria found naturally in soil	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Aspergillus fumigatus*	*Pseudomonas fluorescens*

12	A carrier used in preparation of biofertilizers	Rubber	Peat	Plastic	Soil	Peat
13	A fertilizer consisting of growing plants that are ploughed back into the soil	Green manure	Vermicompost	Biomanure	Organic fertilizer	Green manure
14	Chemoautotrophic involved in nitrification	*Alcaligenes*	*Fusarium*	*Nitrosomonas*	*Arthrobacter*	*Nitrosomonas*
15	Cyanobacteria are	Photoheterotrophs	Chemotrophs	Prototrophs	Photoautotrophs	Photoautotrophs
16	Denitrification is a microbially facilitated process of	Nitrate degradation	Nitrate assimilation	Nitrate oxidation	Nitrate reduction	Nitrate reduction
17	Denitrifying bacteria	*Thiobacillusdenitrificans*	*Bacillus*	*Aspergillus*	*Micrococcus*	Thiobacillusdenitrificans
18	Enzyme involved in phosphate solubilization	Oxidases	Reductases	Kinases	Phytases	Phytases
19	Foliar spray is	Spraying on roots	Spraying on Stem	Spraying on leaves	Spraying on Flowers	Spraying on leaves
20	Indole acetic acid and gibberelins are	Hormones of bacteria	Hormones that retard plant growth	Plant growth hormones	Weedicides	Plant growth hormones
21	Liquid extract of composting by earthworms	Vermiwash	Germiwash	Wormiwash	Liquiwash	Vermiwash
22	Majority of atmospheric nitrogen is obtained from	Fossil fuel	Hospital waste	Sewage waste	Industrial waste	Fossil fuel
23	Microorganisms make soluble phosphate from insoluble phosphate by producing ________	Hydrochloric acid	Sulphuric acid	Nitric acid	Organic acids	Organic acids
24	PGPR is	Phosphorous growth promoting bacteria	Plant ibberellin promoting bacteria	Plant growth promoting biomass	Plant growth promoting bacteria	Plant growth promoting bacteria
25	Phyllosphere refers to	Surface of roots	Surface of leaves	Surface of Stem	Surface of flowers	Surface of leaves
26	Rhizobacteria are bacteria growing in & around ______ of	Leaf	Root	Stem	Fruit	Root

	plants					
27	VAM is	Ventricular arbuscular mycorrhizae	Vesicular augumenting mycorrhizae	Vesicular arbuscular mycorrhizae	Vesicular arbuscular mycobacterium	Vesicular arbuscular mycorrhizae
28	Which are important nutrients for plant growth in soil?	Nitrogen	Phosphorous	NPK	Potassium	NPK
29	Which bacteria is used as biofertilizer in sugarcane crop?	*Beijerinckia*	*Acetobacter diazotrophicus*	*Bacillus*	*Pseudomonas*	*Acetobacter diazotrophicus*
30	Which forms symbiotic relation with higher plants?	*Aspergillus fumigatus*	*Bradyrhizobium*	*Pseudomonas fluorescens*	*Mycorrhizae*	*Mycorrhizae*
31	Expect Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants?	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
32	Rhizobium has symbiotic association with_______	Legumes	non-legume crop	sugarcane	paddy	legumes
33	Which of the following is not the biofertilisers producing bacteria?	Nostoc	Anabena	Both a and	Clostridium	Clostridium
34	Which of the following is capable of oxidising sulfur to sulfates?	Thiobacillus thioxidans	Desulfotomaculu m	Rhodospirilliu m	Rhodomicrobiu m	Thiobacillus thiooxidans
35	Azolla is used as biofertilizer as it has _______	Rizobium	Cyanobacteria	Mycorrhiza	Large quantity of humus	Cyanobacterium
36	The most quickly available surce of nitrogen to plants are______	amide fertilizers	ammonia fertilizers	nitrate fertilizers	ammonia nitrate fertilizer	amide fertilizers
37	Most effective pesticide is _______	carbamates	organophosphates	organochlorine s	phosphates	carbamates

38	Which is true for DDT	not a pollutant	an antibiotic	an antiseptic agent	a non degradable pollutant	a non degradable pollutant
39	Which is major component of bordeaux mixture?	copper sulphate	sodium chloride	calcium chloride	magnesium sulphate	sodium chloride
40	Which one is correctly matched	carbamates-malathion	organophosphates -cabofuran	carbamates-malathion	organochloride-endosulphan	organochloride-endosulphan
41	IPM stands for	integrated plant manufacture	integrated plant management	integrated plant management	integrated pest management	integrated plant management
42	Which is major component of bordeaux mixture?	copper sulphate	sodium chloride	calcium chloride	magnesium sulphate	sodium chloride
43	Insecticides generally attack	respiratory system	muscular system	nervus system	circulatory system	muscular system
44	Organisms associated with sorghum and cotton which provide nutrition to them are	Azospirillum, Azotobacter	Azotobacter, Azospirillum	Anabena, Rhizobium	Rhizobium, Azotobacter	Azotobacter-Azospirillum
45	Azolla as biofertilizer, increase the yield of rice fields by ________	10%	20%	30%	50%	10%
46	Denitrification is __________	reduction of nitrate to nitrogen gas	reduction of nitrate to organic nitrogen compounds	both a and b	reduction of ammonia	Both a and b
47	Which of the following soil microorganism is involved in the reduction of sulfates to	Thiobacillus thiooxidans	Desulfotomaculum	Rhodospirillium	Rhodomicrobium	Desulfotomaculum

	hydrogen sulphide					
48	Which one of the following structure is formed in plant roots by mycorrhizae	Arbuscles	Hartig net	Haustoria	Rhizomorph	Hartig net
49	Except Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants__	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
50	Which one of the following genes is responsible for nod factor in bacteria	fix gene	gag gene	nif gene	nol gene	nol gene
51	In which one of the following relationship one partner benefits but the other is neither hurt nor helpless	Amensalism	Commensalism	Parasitization	Predation	Commensalism
52	The proteinaceous compounds ae converted to ammonia in the presence of which one of the following bacteria	Ammonifying bacteria	Denitrifying bacteria	Nitrifying bacteria	Putrefying bacteria	Ammonifying bacteria
53	In soil, which one of the following bacterial genera is responsible for degradation of cellulose	Escherichia	Pseudomonas	Salmonella	Staphylococcus	Pseudomonas
54	Which one of the following compound is known as the most resisant to microbial degradation during organic matter decomposition	cellulose	chitin	hemicellulose	lignin	lignin
55	Soil microorganisms influence above ground ecosystems by contributing to except which one of the following	plant nutrition and health	soil fertility	soil structure	soil texture	soil texture
56	Mycorrizha is a symbiotic	Crick	Fisher	Frank	Funk	Frank

	association between a fungus and the roots of a vascular plant, was first observed by which one of the following scientist					
57	Denitrification is done only by microorganisms, usually by which one of the following	Facultative anaerobes	obligate aerobe	phototrophic aerobe	Microaerophilic	Facultative anaerobes
58	The plant disease control agents inclde to which one of the following microorganism, except?	Ampelomyces quisqualis	Bacillus subtilis	Trichoderme sp.	Bacillus anthrax	Trichoderma sp.
59	In plants, the strains of which one of the following bacterium initiates to the formation of galls?	Agrobacterium	Rhizobium	Pseudomonas	Ralstonia	Agrobacterium
60	In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules	Bradyrhizobium japonicum	Rhizobium leguminosarum	Sinorhizobium meliloti	Both a and b	Rhizobium leguminosarum
61	Ammonia produced in the bacteriod needs to be transported to the plants through which one of the following membrane	lipid membrane	periplasmic membrane	symbiosome membrane	plasma membrane	symbiosome membrane
62	Pyrethrin is got from	Azardiachta indica	Urtica dioca	Tagetus erecta	Chrsanthemum cinerarifolium	Chrsanthemum cinerarifolium
63	Which one is green manure/biofertilizer	Sesbania	Rice	oat	Maize	Sesbania
64	Azolla is used as biofertilizer as it has ________	Rhizobium	Cyanobacteria	Mycorrhiza	Large quantity of humus	Cyanobacteria
65	Green manuring increases the crop yield by	5-10%	15-25%	30-50%	80-90%	30-50%

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

[18MBP204]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
FIRST INTERNAL ASSESSMENT, JANUARY - 2019
SECOND SEMESTER
MICROBIOLOGY
ENVIRONMENTAL AND AGRICULTURAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**
Date: 05 /02/2019 [AN] **Class: I M.Sc. MB**

PART A – (20 x 1 = 20 marks)
Answer all the questions

1. Copper is used in water treatement as a
   a.Disinfectant b.Indicator c.Coagulant d.Flocculants
2. Bacteriological examination of water usually employs
   a. Total count b.Multiple tube method c.Membrane filters count d.Plate count
3. Which of the following is the type of endosymbiosis
   a.Commensalisms b.cooperation c.mutualism d.predation
4. Microbes in air can be enumerated by
   a.Settle plate method b.Pour plate mehod c.Spread plate method d.Streak plate method
5. 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
6. Most of the indicator oranisms 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
7. The optimum rate of relative humidity for the survival of the most microorganisms is
   a.40-80% b.60-80% c.50-80% d.30-80%
8. ________ is a seasonal disease that can be deadly when becomes pandemic
   a.Tuberculosis b.Leprosy c.Food poisoning d.Influenza
9. __________ is an opportunistic fungal disease of human caused by inhalation of spores
   a.Sporidiasis b.Penicilliosis c.Aspergillosis d.Candidiasis
10. Air doesn't have ___________ flora
   a.Indigenous b.Endogenous c.Exogenous d.Subgenous
11. HEPA is an
   a.Water filter b.Soil filter c.Air filter d.Smoke arrester
12. Human impact on the environment is termed as
   a.Metagenic b.Mutagenic c.Carcinogenic d.Anthropogenic
13. Spores of ______________ travel over thousand kilometers
   a.Blastomyces *b.Fusarium* *c.Puccinia graminis* *d.Aspergillus*
14. The dominant microflora of outside air are
   a.Fungi b.Bacteria c.Algae d.Virus

15. Vapours of _________ are strongly germicidal
a.Poly ethylene glycol b.Propylene glycol c.Glycerol d.Glycerin

16. Which gas dominates composition of air?
a.CO2 b.Oxygen c.Nitrogen d.Hydrogen

17. All are particulate pollutants except
a.dust b.ozone c.soot d.Smoke

18. Air pollution is severe in ____________
a.Cities bAgricultural .field c.Schools d.Desert

19. Algal bloom results in _________
a.Global warming b.Salination c.Eutrophication d.Biomagnification

20. The major pollutant from automobile exhaust is ________
a.NO_2 b.CO c.SO_2 d.Soot

PART B – (03 x 02 = 06 marks)

Answer all Questions

21. What is biochemical oxygen demand?
22. What is a trickling filter?
23. What are electro static precipitator and their uses?

PART C – (03 x 08 = 24 marks)

Answer all questions choosing either a (or) b. (All questions carry equal marks)

24. a) Write the detailed notes about the bacterial examination of water?

(or)

b) Explain the physical, chemical and biological methods of Sewage treatment

25. a) Give a notes on sludge digestion; activated sludge, aerating filters, oxidation pond.

(or)

b) Write a detailed notes on disease caused by air borne microbes

.

26. a) How to enumerate bacteria in air

(or)

b) Write about the air samplers and sampling techniques.