<u>Environmental Biotechnology</u>

Introduction

Environment consists of diverse biotic and abiotic components which helps in functioning of each living organisms. Introduction of pollution into physical and biological components of earth and effects its normal environmental processes. The word “pollution” derived from latin “Pollutioneum” means make dirty or defile. Pollution is in the form of chemical substances, physical from like heat or light and biological organisms. Environmental pollution issues is global problem in both in developed and developing countries due to population growth, urbanizationand industrialization activities.

According to Odum (1971): Pollution is defined as an undesirable modification in physic-chemical and biological characteristics of major environmental compounds like air, water and soil that may harmfully affect the life or create health hazard to living organisms. The pollution causing substances generally called as pollutants and they are in the form of gas, liquid and solid forms. Pollutant are component such as substances, energy, as noise and heat or light. Pollution is often classed as point source or nonpoint source pollution Biotechnology Environmental Biotechnology Types of Environmental Pollution

Types and Classification of Pollution

Pollution are classified depents on its biodegradation nature, medium of occurance and phases of pollution. Following section discuss in details about various types of pollution, its occurance and nature.

i. Classification of pollution based on the biodegrdation nature:

Pollutants are classified in two types biodegradable pollutants and non-biodegradable pollutants based on its nature of degradation by living organisms.

Biodegradable pollutants: Substances or pollutants are the ones that broken down to simple compounds by the action of living organisms, incuding organi waste materials, nitrate, phosphates, and inorganic salts. In this process pollutants are decomposed, removed or used as a nutrient source by various microorganimsms.

Example: Organic materials used by living microorganism to obtain nutrient and energy from carbohydrates and proteins. Therefore biodegradable substances are “temporary nuisance” and it is easily hamless in shorter time. These biodegradable substances also called as rapidly degradable or non-persistent pollutant.

Non-degradable pollutants are substances which cannot be converted into simpler or harmless substances by living organisms. The wide range of non-biodegrable polluting substances are reased into environment such as polythene, insecticides, pesticides, metals like lead, arsenic, radioactive subtances, synthetic fibers, glass and iron etc. These pollutnats are extremely persist in ecological system for long period of time.

ii. Classification of pollutants based on its phases:

Depending on the nature of pollutants and their interation with environmental process it is classified into gas, liquid, solid and waste withour weight. Liquid waste are mainly wastewater from various industries, commercial and household establishment. Gaseous pollutants are CO,CO_2,SO_2,NO_2 and O_3. Solid waste includes garbages and industrial ashes. Waste without weight are mainly radioactive elements, heat and Biotechnology Environmental Biotechnology Types of Environmental Pollution noise. These all form of pollutants indent to interact each other and cause different harmfiul impact on the environment.

iii. Classification: Medium of occurrence Environmental pollution are classified based on the part of environment (medium) in which they exisit or affect and results which particular pollution causes. This is important classification to understand the nature and its cansequences of pollutants on environment including hman, plants and animals.

Major Types of pollution: o Air pollution o Water pollution o Soil pollution o Noise pollution o Radiation pollution o Thermal pollution o Light pollution

i. **Air pollution :**

Air pollution refers to the release of pollutants into the air that are detrimental to human health. Harmful gases, dust, smoke enters into the atmosphere. Natural and anthropogenic sources of pollution. Air pollution emitted from point source of pollution such as smoke stacks and non-point source of pollution like cars and factory. Primary air pollutants are directly emitted from a process such as volcanic eruption and industrial stacks whereas secondary pollutants are formed by th results of primary pollutants react or interact (e.g: Photochemical smog). Biotechnology Environmental Biotechnology Types of Environmental Pollution Wide range of air pollutants are released into atmosphere due to natural and man made activities. Gaseous pollutants like Sulfur dioxide (SO_2), Caron di-oxide (CO_2), Nitrogen oxides (NO_x), Ozone (O_3), Carbon monoxide (CO), Volatile organic compounds (VOC), Hydrogen sulfide (H_2S), Hydrogen fluoride (HF) and Gaseous forms of metals are released into atmosphere. The sources of includes Fossil smelters, fuel plants, industrial facilities, oil and petroleum refineries, and manufacturing facilities as well as and auomobile sources.

Transport and diffusion of Air pollutants Biotechnology Environmental Biotechnology Types of Environmental Pollution

Primary air pollutants

Particulates are also called as particulate matter (PM) referred as particles in atmosphere. This is tiny solid or liquid particle suspended in air. Particulates matter are obvious from of pollutants small than 100 μm in diameter whereas 0.001 to 10 μm pollutant particles in the atmosphere is called as suspended particle occurs in near pollution such as urban, industrial, commercial as well as power plant. Occurance of these particle in air linked with health problem like lung cancer and heart disease.

Sulphur oxides (SOx): There are various natural and man-made action responsible for release of SOx in to atmosphere. Main natural sources are volcanoes. Other sources like coal and petroleum products also responsible for release of sulphur containing compounds. Nitrogen **oxides (NOx) :**

NOx pollutant generally emitted from combustion process and causes photochemical smong and acid rain.

Carbon monoxide : This very commonly emitted pollutant released activities like fromvehicle exhaut, incomplete incineration, natural gas, coal and wood.

Carbon dioxide (CO2): It is a naturally occurring gas emitted from various incineration and other natural process.

Volatile organic compounds: One of the most emitted outdoor pollutant , divited in two types such as methane (CH_4) and non-methane (NMVOCs). Methane gas one of the greenhouse and extremely contributes to enhanced global warming. Other air pollutions are persistent free airborne fine radicals, toxic metals like mercury and lead. Ammonia also emitted as a result of agricultural processes.

Air Pollution status in India

In India air quality level often exceeds the National Ambient Air Quality Stadards (NAAQS) due to uncontrolled release of various polluatnts like particulate matter (PM), sulfur dioxide (SO_2), nitrogen oxides (NOx), carbon monoxide (CO), and ozone (O_3). As per the report released by World Health Organization (WHO), around 37 cities from India feature in the top 100 world cities with the worst PM10 pollution, and the cities of Delhi, Raipur, Gwalior, and Lucknow are listed in the top 10. Main sources are fuelwood and biomass burning, fuel adulteration, vehicle emission and traffic congestion and large scale crop residue burning in agriculture fields.

Methods of monitoring air pollution

Most frequently occurring pollutants in an urban environment are particulate matters, respirable suspended particulate matter RSPM. carbon monoxide (CO), hydrocarbons (HC), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3) and photochemical oxidants. The air quality generally measured using wide range of methods. In point sources, pollutants like SOx , NOx, CO and PM are measured whereas in ambient air pollutants like SOx , NOx, CO, SPM, Hydrocarbons and Ozone are measured. Particulate matter dust sampler are used to collect and measure the particulate matter in secified location for the defined time period. To measure the gaseous pollutants, Respirable Dust (PM 10) Samplers and fine Particulate (PM 2.5) Samplers are used. This ampler used to do sampling of gases like SO_2, NO_2, Cl_2, H_2S, O_3, NH_3 etc using wet chemical methods.

ii. Water Pollution :

Water is a essential element of life and also important natural resource. Two third of earth occupied with water resources. Around 98% of water resource is in the form of seawater and only 2% is seawater is fresh water,in which 1.6 % locked up in polar ice caps and glaciers. Only another 0.36 % is found underground in aquifers and wells. Therefore, only about 0.036% of the planet's total water supply is accessible in lakes and rivers. Water pollution is due to presence of chemical or biological substances (pollutants) in excessive quantity which leads to adverse effect to living organisms. Water quality affected by human activity and is declining due to urbanization, industrial production, population growth and climate change. Water pollution is due to point source pollution and non-point source pollution. Point sources of contaminants that enter a waterway through a discrete conveyance, such as a pipe or ditch like sewage treatment plant, a factory, or a city storm drain. Whereas non-point source of pollution diffuse contamination that does not originate from a single discrete source. NPS pollution is often the cumulative effect of small amounts of contaminants gathered from a large area. The leaching out of nitrogen compounds from agricultural land which has been fertilized is a typical example. Main water polluting sources are

i. Domestic wastewater and effluent
ii. Industrial effluent sources
iii. Agricultural activities
iv. Radioactive wastes and
v. Oil spills. Biotechnology Environmental Biotechnology Types of Environmental Pollution

i. Domestic wastewater and effluent It is generated due to domestic activities like drinking, cooking, bathing, claning and other household activities. Around 70 to 805 of this discharge in to municipal drains and ultimately reach to river and lakes. This is main source of water pollution and causes oxygen demand in freshwater system and affect the balance of aquatic system. Municipal wastewater is considered as a main source of pollution due to particial or un-treatment of wastewater before they release to freshwater specifically countries like India.

ii. Industrial wastewater Developing counties like India, industrial activities are high and a wide range of waste products generated from industrial discharges. Dominating industries are textile, paper manufacturing , metal work, petrochemical and refining, food processing, distillery and chemicals. Most of the Indian rivers are polluted with industrial effeluents. For instances, the river Ganga, polluted with various industrial effluent discharge due to partial or untreated effluent from chemical, textile dying, tanning, pulp and paper, petrochemical, rubber, fertiliser and other industries are located in bank of river. Even Ganga Action Plan, to control water pollution, has failed miserably.

Agricultural activities Pollutants like fertilisers, insecticides and pesticides, farm animal wastes and sediments are released to nearby water bodies due to agricultural activities. Execessive nutrient like nitrate and phosphate causes eutrophication in lakes and other water bodies. The nitrates, when mixed with water, may cause methemoglobinemia in infants. Uncontrolled use of various insecticide and pesticides in agricultural is also one of the major source of water pollution. High level of organic waste from animal farms leads to release of odour and pathogenic microorganisms.

As a results of various commercial, household and industrial activities waterbodies are receiving large quantities of pollutnats like plastic waste, chemicals, pharmaceuticals, pesticides, fertilizers and detergents (Nitrogen and Pharphorus). Generlaly these pollutants are catagoried into 3 types biological, nutrients and physical pollution. Biological pollution are infectious pathogens, oxygen demanding waste and chemicals. Nutrients includes Biotechnology Environmental Biotechnology Types of Environmental

Pollution fertilizers, toxic inorganic materials and persistent organic pollutants. Physical pollutants are sediments, thermal Pollutants and solid wastes.

3. Soil Pollution :

Soil pollution is defined as the occurance of various toxic contaminants which leads to harmful effect on human health and ecosystem. There are two main causes through which soil pollution is generated: anthropogenic (man-made) causes and natural causes. Soil contamiannats are heavy metals, inorganic salts like phosphates, sulfates, nitrates and organic compounds like lipids, proteins, hydrocarbons, PAHs and alcohols. Most of this compounds are formed due to soil microbial activities like decomposition of organisms (e.g., plants and animals). Other causesof soil pollution includes pollutants from atmosphere into soil and from surface water bodies and shallow groundwater flowing through the soil.

Sources of soil pollution Soil generally affected with naturally occurring contaminants and anthropogenic pollutants. Soil pollution mainly occurs due to the following: industrial wastes, urban wastes, agricultural practices and radioactive pollutants and biological agents. Industrial activity has been one of the major contributor to the soil pollution particularly from mining and manufacturing sectors. Oil spill also contribute significant soil pollution. Agricultural Activities like chemical fertilizer and pesticide application in modern agricultural activities. As a outcome, the chemicals enter in to groundwater, surface water and finally mix with soil and reduce the fertility of the soil. Waste Disposal is one of the major growing concern, as the increase in population density pose increase waste generation. Even the sewer system ends at the landfill, where the biological waste pollutes the soil and water. Accidental Oil Spills also contribute in soil pollution. The hydrocarbon adversely affect the soil fertility. Oil spill generally happen during storage and transport of chemicals. Oil spill also further causes grund water pollution through soil. These pollutants affect and alter the chemical and biological properties of soil. As a result, hazardous chemicals can enter into human food chain from the soil or water, disturb the biochemical process and finally lead to serious effects on living organisms.

Water and soil pollution assessment methods

There are international and national standardized methods. Pollutants can be analyzed by – Physical, Chemical, and Biological methods. Physical tests include solid concentration and Biotechnology Environmental Biotechnology Types of Environmental Pollution turbidity of water. Chemical methods of analysis include pH measurement, nutrients in water, toxic metals in water, biological oxygen demand and chemical oxygen demand. Biological methods include use of plant, animal, bacteria as an indicator of water pollution

4. Noise pollution:

Disturbing or excessive noise that may harm the activity or balance of human or animal life. The source of most outdoor noise worldwide is mainly caused by achines , transportation systems, motor vehicles engines, aircraft and train and industrial operations. Noise pollution – Causes: Outdoor noise can be caused by machines, construction activities, and music performances, especially in some workplaces. Noise-induced hearing loss can be caused by Outside (e.g. trains) and indoor activities like music noise. Hamful effect of noise pollution Level in db Effects Up to 23 No disturbance 30-60 Stress, tension, psychological illness,heart attack 60-90 Damage to health, psychological and vegetative (disturbance in stomach-gall function,pain in muscles, high blood pressure, disturbance in sleeping) 60-120 Damage to health and ontological (ear disease) effects Above 120 Painful effects in long term This affects human health, comfort and efficiency. It causes muscles to contract leading to nervous breakdown, tension and affects health efficiency and behavior. In addition to serious loss of hearing due to excessive noise, impulsive noise also causes psychological and pathological disorders. Brain is also adversely affected by loud and sudden noise as that of jet and aero plane noise.

5. Thermal Pollution

Increase in temperature of water leads to harmful effect on human, animal and aquatic system and causes significant changes in the aquatic communities in the water by the activities of industriels. Majority of thermal and electric power industry discharge significant quantity of hot effluent and wastewater in to adjacent river and waterbodies. Major sources of thermal pollution includes nuclear power plants, coal fired power plants, industrial effluents, domestic sewage and Hydro-electric power plant. Biotechnology Environmental Biotechnology Types of Environmental Pollution The effect of thermal pollutants includes reduction in dissolved oxygen of water, increase in toxicity, interference with biological activities, interference with reproduction, direct mortality and food storage for fish. Aquatic communities like fishes, trout and walleyes required a minimum level of dissolved oxygen concentration (4 mg/l), When the temperature of the receiving water is raised, the dissolved oxygen level decreases and the demand for oxygen increases and cause death of aquatic organisms. To control the thermalpollution, several industries have cooling towers, where the heated water is cooled.

Introduction:

POLLUTION MONITORING – A GENERAL BIOASSAY

It has been realized by the environmental protection agencies (EPAs) world over that this new alternate technology holds tremendous potential in the restoration of environmental quality and its management.

The diverse techniques availability now has placed toxicologists and environmental scientist in a better position in order to scrutinize the environmental health from diagnostic, preventive and remedial points of view.

However, till recent years the conventional surveillance was largely based on physical and chemical methodologies. But in any pollution study, knowledge of the mechanism of biological response plays a crucial role as the ultimate damage is affected on the living system.

Moreover, it often becomes difficult to detect the intermittent and chronic pollution effects through conventional technologies.

Bioassays do not require assumptions and it provide the means of quickly viewing samples for toxicity assessment.· Thus bio-monitoring can serve in a corresponding fashion with other strategies in defining toxicity threshold.

In this regard, numerous groups of plants, microbes and animal .test samples have been utilized at different group levels.

Choice of Criteria or Parameters

There are some parameters which are essential in bioassay studies as to the reliability, predictivity and universal acceptance. However, the techniques of bioassay largely encompass three types of criteria, viz. symptoms of visual damage, genotoxicity test and assessment of the responses at the metabolic levels.

Visual rating:

At the initial stages of bioassay, it was largely based on visual rating only. Hence the concept of LD50 or LC50 emerged for fishes, i.e. the dose at which 50% of test organism is affected. Computer programmes for the determination of these values have also been developed The growth rate, productivity or phenology are considered. For the microbial growth, turbidometric analysis or electronic particle counters are used more often nowadays. In case of higher plants seed viability, germination frequency, growth rate of different parts and any visual damage symptom of leaves and other parts are considered. Presence or absence of a particular species in a polluted environment (indicator) is also another good criterion. However, no single indicator species can fulfil all the purposes equally well. Hence multi species testing concept has emerged.

Genotoxicity test :

This involves the evaluation of damage at the cellular and sub-cellular levels. The primary sites of interaction are the cells between chemicals and biological systems. At this level the importance of toxicity assay depends on the hypothesis that cellular changes are finally reflected in the metabolic and morphological disorders at the organismic, species or population levels .At the cellular level include different biomolecules, organelles, immune reactions (immunoassay) and membrane processes. These involve simple and inexpensive tests. **Cytotoxic tests** depend on the estimation of chromosome damage including breakages, sister chromatid exchange (SCE) or micronuclei counting. Disfunction of plasma membrane is mainly caused by lipid peroxidation affecting its fluidity and structural integrity. It can be measured by conductivity studies indicating ion leakage or tryphan blue uptake or leakage of enzyme like LDH (lactose dehydrogenase). Recently DNA probe has been considered as a outcome to these parameters, which is helpful in the identification of pathogens in water samples.

Metabolic rating

With the advancement of knowledge of the physiological and biochemical mechanisms of metabolic processes, it was thought worthwhile to consider the changes in some of these parameters induced by pollution stress as unquestionable 'biomarkers'.

Both qualitative and quantitative assessment of these markers are involved in biomonitoring. They include changes in the contents of chlorophyll molecule and its fluorescence kinetics contents of soluble protein; nucleic acids; changes in the activities of key enzymes, their reaction kinetics and electrophoretic assessment of isozymes or the induction of some stress proteins.

Examples Highlighting the Utility of Bioassay

Plant test systems - Among the test systems in plant groups, algal bioassays have been used since 1970s. This is due to their high sensitivity, easy availability and culturing facilities in limited areas.

They are considered as good indicators of pollution. The primary criteria considered are growth rate, productivity (biomass yield) and LC50 values.

At the metabolic level, the photosynthetic efficiency has also been taken into account using C14 assimilation, O_2 evolution and by noting the degradation of in vivo chlorophyll fluorescence. Inhibition of some key enzymes has been considered as well.

Some of the algae, commonly considered as test materials, are Chlorella, Scenedesmus, Selenastrum, Navicula, Spirulina, Anabaena and Microcystis among the microalgae, while among the marine macroalgae.

In case of marine macroalgae, changes in frond structure and biomass have been used as pollution detection criteria. Benthic community diversity has also been considered.

Algal species are often used as bio-indicators of pollution. Dominance of Cyanophyceae (blue-green algal group) in the phytoplankton community of a water body indicates its eutrophic nature.

While the bacterium Salmonella has been used in Ames Test to detect atmospheric mutagens with the help of its different mutant strains. Bacteria are good indicators of organic pollution.

Again the phenomenon of bacterial bioluminescence has been used as an indicator in the analysis of atmospheric gases and other compounds like, formaldehyde, ethyl acetate etc.

Bioluminescence is an enzymatically catalyzed light-emitting reaction in living cells. Photobacterium phosphoreum colonies are used in a special photodetector, where change in the emission of light due to pollutant effect is detected by a sensor, then amplified and recorded in a computer .

Lichens (which represent commensalism between algae and fungi) are now commonly used in 'mapping' cities for atmospheric S02 detection.

Animal test systems

Among the faunal species, toxicity to fishes have been considered since 1950sas a good criterion of xenobiotic bioassay to provide the basis for strategy formulation in the release of toxicants. It has provided the basic tool in monitoring pesticides, phenolic compounds and wastes from tanneries and textile industries.

In fact, the concept of LD50 first emerged from studies on fishes. Toxicity assessment has been done on morphology, behaviour, changes in different organs, muscles and metabolism as well.

Lately inhibitory effects of xenobiotics on fishes have been investigated on the enzyme AChE (acetylcholine esterase), which is a neurotransmitter and has been found to be a good marker of pesticide pollution.

Some of the common fish types in bioassays are Catla, Labeo, Channa, Teleost and Tilapia. Other faunal groups include Protozoa, particularly the ciliated ones, as good bioindicatorsthrough changes in their behavioural patterns, and thus the concept of ethogram has developed.

Rotifers are a kind of helminths among the zooplanktons and grow mainly among aquatic vegetation. They constitute another class of fauna which have proved to be good indicators of saprobity and trophic levels of water.

The rotifers in biomonitoring of water have advantage lies in their easy recognition, easy cultivation, slow growth rate and year-round availability.

CELL BIOLOGY IN ENVIRONMENTAL MONITORING

Cell Biology is a specific branch of Biology surrounding the structural and functional aspects of cells and their components.

It is being effectively used as an important parameter of screening environmental mutagens and carcinogens.

Cell Biological Methods

Cells are the sites of primary interaction between toxicants and biological systems and cell biological methods act as 'biological dosimeters' at cellular level damages.

The tests of cell biology intend to trace the damages caused by different environmental toxicants on different cellular parameters like cell membrane, cell organelles and chromosomes, carrying the genetic materials like nucleic acids (DNA &RNA) and protein.

They help in understanding the basic mechanism of toxicity, mutagenecity and carcinogenicity.

It got a major thrust with the exponential growth of industrial chemical production and realization of their effects on human genetic system.

Like any other scientific discipline, mere identification of the phenomenon led to the technological 'innovations of detection at both in vitro and in vivo levels to find out the remedial measures.

Test Systems for Assessment of Genetic Damage

Various test systems have been developed using microorganisms, plants, laboratory animals and human cell cultures to assess the toxic and mutagenic potency of numerous chemicals and radiations and even 'living mutagens' or virus and bacteria-induced mutations.

1. Ames test: The tests based on cell-biological principles include the classical Ames Test. It involves the utilization of a series of strains of the bacterium Salmonella typhimurium with the specific ability to detect certain types of chemical mutagens.

It is a very widely used preliminary short-term testing and screening of drugs, agricultural chemicals, cosmetics, food additives, inorganic metals and other pollutants. It is based on the principle of the frequency of reversion of mutations at the histamine locus (His- to His+). Common yeast cells (Saccharomyces cerevisaei have also been used for such type of detections.

2. Cytogenetic assay:

In higher plants and animals detection of permanent genetic damages takes longer time depending on the life-span. Thus in these cases chemical induceddisruptions in the chromosome system, be it in vegetative or reproductive cell, is taken as the criterion of assessment of mutagenecity or carcinogenecity, as they constitute the basic hereditary materials. Other plant systems that have helped reliable screening and monitoring hazardous environmental chemicals are pea, maize, soybean, Crepis and Tradescantia. Tradescantia has unique characteristics which provide two ideal genetic endpoints for mutagenecity testing, viz. 'stamen hair' assay and micronucleus test. The stamen hairs are highly sensitive to gaseous chemicals like Freon-22, Benzene, Ozone, $N0_2$, $S0_2$ and they serve as indicators by changing their colour
(i) Chromosome damage: The most common method of toxicity testing of chemicals is the so called 'Allium test or the use of broad bean Vicia faba to check the extent of induction of chromosome aberrations in form and behaviour.

These may be of different types including fragmentation of chromosomes, bridge formation and disruption of the normal cell division.

They serve as clear 'hazard indicators' and can even be quantified. The severity of the damage may depend on the chemical make-up of the toxicant.

(ii)Micronucleus test (MNT): This is another method where very small nuclei are scored within the cell, formed by large-scale fragmentation of chromosomes. The extent of such nuclei (MN) formation can be directly correlated to the severity of damage. This is now routinely used for screening mutagenic compounds due to relative simplicity and rapidity. It is most practical for analysis of bone-marrow cells of animals in vivo after exposure to chemicals.

(iii) Sister-chromatid exchange (SCE): This is an effective parameter of cytogenetic assay. It is based on the damage caused to DNA molecule and misexchange of chromosome segments (Fig. 6). This is characteristically revealed first by using Bromodeoxyuridine (Brd-U) dye and then stained with a fluorescent dye technique. The potentiality of this test is that even after a short exposure it can be easily detected.

3. Membrane damage :

Besides the aforementioned nuclear parameters, damages to cell membrane and cellular organelles can be detected in a number of ways. The plasma membrane is the first structure encountered by a toxic agent upon reaching the cell.

The membranes are the sites of uptake, deposition and elimination of chemicals. The lipoprotein composition of the membrane act as selective barrier due to its semi-permeable nature.

Many toxic compounds can modify the membrane structure and its fluidity by the so-called 'lipid peroxidation', i.e. oxidative destruction of the membrane.

Dysfunction of the membrane can ultimately lead to cell and organismic death. Inside the cells, other membrane systems including those of mitochondria, nucleus, vocuoles, lysosomes, etc. act as effective sites of compartmentalization.

MOLECULAR BIOLOGY IN ENVIRONMENTAL MONITORING

In the monitoring of environment, molecular probe is primarily based on small segment (oligonucleotide) of Nucleic acid (DNA or RNA) capable of recognizing complementary base sequence in the target DNA from the sample organism (DNA probe).

It could also be through the sequence recognition of specific protein (Antigen-Antibody), as is done in immunoassay. DNA probe or DNA-gene probe as it is called, helps in the detection of the gene rather than its product. While immunoassay identifies the amplified gene product.

These recently developed molecular probes are utilized in various biotechnological studies. In the arena of environmental analysis, these are very useful in the diagnosis of infectious diseases, identification of waterborne pathogens, microbes in mixed cultures and food contaminants. They are very sensitive, accurate and faster too. While the conventional tests, based on morphology and physiology, may take a few (4-5) days, the molecular technique takes only a few hours.

Immunoassay :

Immunological techniques based on antigen-antibody (protein) reactions are now widely adopted in various environmental analysis including diverse pesticides/herbicides, and identification of microbial pathogens and their toxins in food and water. They are useful only in those cases where substances show immunological properties.

Of these techniques, both enzyme immunoassay (EIA) and latex agglutination tests are widely applied.

Enzyme immunoassay relies upon an enzyme tag, where an enzyme is used as a marker, viz. Horse Radish peroxidase, Glucooxidase and Alkaline phosphatase.

They are covalently bound to a ligand (antibody protein). In recent years the use of monoclonal antibody (MAb) which is a homogeneous antibody derived from single clone of cells, has made the test more specific, sensitive and time saving too.

BIOSENSOR IN ENVIRONMENTAL ANALYSIS

Biosensor is an analytical device based on the sensing response of biological materials (immobilized) in combination with an electro-chemical transducer to convert the biological reaction into a digital electronic signal which is proportional to the concentration of the target substance (analyte). It is highly specific and accurate in detection.

The 'enzyme electrode,' however, was christened. Since then, it has gained popularity and a vast array of sensors has been developed based on different biodetecting elements.

They are, however, now finding more and more application in the realms o environmental monitoring and pollution detection.

A biosensor essentially comprises three components:

(a)The biological component consists of immobilized biological sensing materials, viz. enzymes, immune-agents (antibody/antigen), lectin, DNA, whole microbial cell, and higherplant or animal tissue slices. The advantage being conferred by biological material is the ability to operate at ambient temperature.

(b) The physical component is a transducing element which converts biochemical interactions into electrical or optical signal and it could be made of platinum, gold or graphite.

(c) While the interface is a polymeric film or membrane linking the biological component to the transducer.

Sensors could be of indirect or direct type. In the former two electrodes (sensing and reference) are used.

Whereas in the latter, the biological component is directly placed on the electrode offering more sensitivity and accuracy as the electrons are transferred from the analyte to the enzyme or other sensing element, and then directly to the electrode.

Sensors, whether enzyme or whole cell, mainly work on the principle of gas electrodes, viz. O2, CO2, H_20, NH_3, H_2S, etc.

Introduction:

BIOTECHNOLOGY IN THE REDUCTION OF CO_2 EMISSION

Elevation in the amount of CO_2 has been seen in the atmosphere which has grown to be a world' biggest problem which is most likely related with the raise in atmospheric temperature which is called as 'Green house effect'. Main cause of green house effect is carbon dioxide, methane, CFC (chlorofluorocarbon) and water vapour. Among these gases, CO_2 is the principal agent and also it is believed that since last 150 years, its level has augmented by about 25%.

Effect of green house effect:

Increase in the of water levels

Change in the rain-fall pattern

Change in the pattern of crops in agriculture

Such changes in the environmental conditions are the main reason for the reduction of carbon dioxide.

These changes can be achieved by chemical phenomena and the opportunity of biotechnological decline is also being concern of and investigated in unlike parts of the world as an exchange approach.

Photosynthesis as a Means of Reducing CO_2 Emissions

Photosynthesis by plants is considered to be an obvious means of energy efficient biotechnological reduction of CO_2 released from industrial exhaust.

The process can be summarized as:

$$nCO_2+2nH_2O \text{ -----} 6(CH_2O)+nO_2+nH_2O$$

In this respect the photosynthetic efficiency of both terrestrial and aquatic vegetation can be augmented.

1 Higher plant photosynthesis:

Rampant deforestation has resulted in the significant reduction of CO_2 sink. It has been estimated that about 1% of tropical rainforests are getting diminished every year. Their conservation is an urgent need and for that matter, two approaches may be made.

On the one hand, selection of fast-growing trees and their propagation is being done and on the other, resource to biotechnological methods like 'micropropagation' and 'synthetic seed' production through tissue cultures are attempted at different centres.

Another biotechnological approach for genetic improvement of photosynthetic CO_2 fixation is also desirable. It is known that the enzyme ribulose-biphosphate carboxylase is the one responsible for CO_2 fixation by plants.

Its two sub-units (large and small) are genetically controlled by nuclear and cytoplasmic genes. Attempts are already being made to genetically manipulate this enzyme in crop plants to increase the photosynthetic efficiency. Same approach could be made in forest trees at the tissue culture level before their propagation, so that regenerated plants from culture would have more CO_2 fixation potential.

2 Microalgal photosynthesis:

Microalgae photosynthesis is a proficient process for CO_2 decline, if strong planned photo bioreactor could be made for their severe crop growing. Owing to their elevated velocity of photosynthesis, they emerge to be superior candidates in this value. The photosynthetic proportion, which is a ratio of the liberate of O_2 to CO_2 permanent, is commonly greater than 1 for microalgae.

This is the phenomena where high amount of O_2 is generated as compared to amount of CO_2 utilized per unit biomass in higher plants. In addition microalgae suggest economics of extent, as they can he developed by quantity as an alternative of region.

So photobioreactors would be useful where cultivation can be made in cultures with artificial light and fibre optic light radiator. Several such reactor design have been proposed and large scale continuous cultivations have been done with Chlorella pyrenoidosa and Spirulina maxima.

An algal culture volume of 15 x 104 m3 can be produced from power plant of 150 MW energy can produced approximate concentration of CO_2.

Moreover, such a huge microalgal biomass could be effectively utilized for extraction of protein as food and feed. It is now known that both Chlorella and Spirulina contain highest amount of protein per unit biomass among the plant kingdom.

CO_2 which are produced from the industries is to a great extent elevated (20%) than its normal altitude in the air. CO_2 drop as of such high amount would thus need algal forms or strains which can tolerate high CO_2 level, e.g. increased alcohol tolerance by yeasts.

The preparation of EMS (ethylmethane sulphonate) or NMU (nitrosomethyl urea) and some more are genetically manipulated chemical mutagens which are producing large amount of CO_2 tolerant mutants in Anacystis nidulans and Synechococcus is cheering.That's why it is identified as known cause of the discovery of large amount of strains, with huge CO_2 easiness, of various algae such as Chlorococcum and Oocystis .

On the other hand application of gene cloning phenomena (genetic engineering) it may possibly to build up strains through more rapidly growth rate to produce huge amount of biomass of the concerned algae.

Reducing CO_2 Emission from Sea Water through biological Calcification

Deep sea offers viable reservoir for long-term storage of CO_2.

This is achieved with the help of calcifying organisms like corals and a number of calcareous green and red algae. They live in a symbiotic relationship and the calcification process is rapid. If algal species can be found out which would tolerate high pH and carbonate, then photosynthesis by such algae may lead to precipitation of $CaCO_3$.

ALGAL PHOTOSYNTHESIS IN WASTE WATER TREATMENT

Waste waters, resulting from human activities, are of municipal, agricultural and industrial origin. Because of the diversity of sources of these wastes, their characteristics also differ extremely.

Primarily they contain organic and inorganic substances, pathogenic organisms and various toxic materials like heavy metals. So the treatment of such wastes for purification is rather complex.

The treatment processes may be principally categorized as physical and biological. The physical process consists of straining and settling. This cannot remove a major portion of organic matter, which remain as colloidal and as dissolved solids.

This can, however, be achieved by what is called the phenomenon of mineralization of organic matter, i.e. breaking down into simpler forms through bacterial oxidation or biodegradation.

This process requires steady supply of oxygen as respiratory requirements of the bacterial flora in the waste .

In conventional biological practices, the oxygen is supplied artificially by continuous 'bubbling' of atmospheric air.

Aerobic oxidation ponds or stabilization ponds are used throughout the world for such treatment. But it needs costly equipments and manpower.

However, it was long known that simple micro-algae can serve as rich source of oxygen in fish ponds. The algal system can supply the oxygen needed by the bacteria and protozoa in the process of degradation of organic contents of waste water. It is a natural process and therefore no artificial aeration is necessary.

The conventional treatment of sewage and other waste waters consists of three phases, viz. primary, secondary and tertiary.

The algal treatment is introduced at the secondary stage where liquid effluent from the primary stage is taken care of and bio-oxidation takes place.

Oxygen Production by Algae :

The process of photosynthesis is known as oxygen generator, where CO_2 and H_20 are used by plants to build carbohydrates and release O_2 in presence of sunlight. The photosynthetic efficiency of many micro-algae is very high.

Under satisfactory environmental conditions with availability of sufficient sunlight, as in many tropical countries; the oxygen produced by these algae surpasses their own respiratory requirement. This surplus oxygen can be made available in oxidation pond by the culture of specific algae. It is an inexpensive and natural process.

The species of different algal genera which are best suited for the purpose are mostly members of the green algae or Chlorophyceae. Some of them are Chlorella, Scenedesmus, Euglena, Chlamydomonas, Hydrodictyon, Ulothrix and Tribonema.

They have high 'oxygen donating' power and are very useful in the biotreatment of waste water. This, however, depends on steady flow of CO_2 in the pond water. It has been estimated that 10-20 ppm of CO_2 is optimum for efficient O_2 donation.

Again, in a carbon-limited system, the pH may get increased up to 10, while the optimum pH for the bacterial growth and action is about 8.3. So a balance is necessary for the proper symbiotic function of the algae-bacteria system.High-rate ponds with less depth (l metre) and with continual stirring is more suitable than facultative ponds.

Oxygen production occurs concurrently with algal production. On the basis of weight, the oxygen production is 1.3 times the algal production. This has been termed as oxygen-algal quotient (OAQ).

This oxygen is valuable for meeting the BOD (biological oxygen demand) requirement of waste water arising out of vigorous respiration of the bacterial flora (decomposers).

The efficiency of the purification process is judged by BOD removal capacity and the ultimate BOD of waste water should not exceed the oxygen produced by algal photosynthesis.

The production and consumption of oxygen should be balanced to ensure that some amount of DO (dissolved oxygen) is present at all times in the effluent.

Besides purification of waste water, such pond could be a source of valuable byproducts. Algal cells rich in protein could serve as food and feed or be used as manure.

Being rich in DO, such ponds may well support fish life, and the algal cells can serve as fish food.

It could also be a means of recovering metals present in the waste, as contaminant, by way of algal cell surface adsorption.

Thus the whole process of algal treatment of waste water is useful in various ways, apart from the primary objective of purification and possible reuse of such water.

This system also reduces the risk of large number of pathogenic microbes getting into the effluent as many of them either become dead and get settled at the bottom of the pond or, are screened out along with the removal of algal biomass.

EUTROPHICATION, ALGAL BLOOMS AND BIOLOGICAL PHOSPHORUS REMOVAL

Eutrophication : The term 'eutrophication' is derived from Greek word meaning "rich", while eutrophic water refers to the stage at which it is enriched with undesirable organic or inorganic nutrients containing phosphorus and to a lesser extent, nitrogen.

Natural sources are nitrogen and phosphorus cycles and man made sources are agricultural and municipal wastes containing fertilizers and detergents as causal agents.

Undesirable amount of these elements in waste water favour excessive algal growth and thus lead to eutrophication. It causes depletion of oxygen, foul smell through generation of sulphides and death of nonresistant organisms.

It results in the colonization of such water by a particular group of organism, cause sedimentation and eventually gets filled with the resistant types. Such a situation has been termed as 'water bloom'. Dissolved organic compounds act as direct source of nutrient for huge algal growth or through bacterial activities release more CO2 favouring algal photosynthesis.

Among the algal species, members of microalgae, e.g. Cyanophyceae (Blue-green algae) are predominant. Different species of the genera like Lyngbia, Microcystis, Anabaena, Aphanizomeum and Oscillatoria are involved.

At least 20 algal species are capable of forming 'blooms'. These species have gas vacuoles in their cells which help them to float, and survive for varying periods of time.

In a dense bloom, gradual oxygen deficiency results in its collapse.' This leads to further release of nitrogen and phosphorus and helps in the development of another bloom. It has been termed as bloom succession. In a mature bloom with excessive growth of the cyanophycean alga, Microcystis aeruginosa, a redish scum is produced.

Seeing the scum from a distance, one can predict that the lake or pond has undesirable amount of phosphorus. Thus it serves as an indicator species. In coastal areas the so-called 'brown tide' is caused by a goldenbrown alga called Aureococcus.

Algae absorb phosphorus and store.it as polyphosphates within the cells. Phosphorus concentration above 6 ppm favour explosive growth.

It has become a, global problem as ponds and lakes gradually turn into marshes, which is why in many western countries, phosphorus-based detergent manufacture is encouragedAlgae are considered to be a biological means of Nz and P3 removal. which in tum also helps checking eutrophication.

Apart from Cyanophyceae. dinoflagellates like Ceratium and Cryptomonas are also present in blooms. In marine condition. dinoflagellates cause red, or brown colouration of the sea.

Centric diatoms and seaweeds like Ulva, Enteromorpha, Cladophora, Gelidium, etc. have also been used for assessing eutrophication of water in marine environment.

Control of algae in eutrophic waters can be done chemically or biologically. Chemical means constitute the use of algicides like copper sulphate, sodium arsenate, 2,4-D (dichlorophenoxyacetic acid) and 2,3-DNQ (dichloronapthoquinone). But all these increase the sludge volume from sedimentation.

Although these viruses, being specific for BGA (bluegreen algae), and do not pose problem to other aquatic organisms yet the dead algal cells may release toxins in water.

Biological Phosphorus Removal from Waste Water:

As phosphorus has ecologically major role in algal yield its elimination from marine bodies is necessary to defend them from eutrophication. Common tradition is to precipitate them chemically with salts of Ca, Fe, Al and Mg with calcium salts. Phosphorus is precipitated as hydroxyapatite.

On the other hand the various processes are the natural one, where phosphate metabolizing microorganisms help out in the procedure. The energy requisite for this is completed through discharge of phosphorus attached as polyphosphate in granules in the bacterial protoplasm.

The bio-P elimination has the benefit that accompaniments of chemicals can be avoided and it helps in the decrease in of sludge volumes.

The principle of biological techniques plant in the disclosure of the organisms to other different anaerobic and aerobic situation. This can be attained with or without nitrogen elimination. When nitrogen elimination is necessary, an anoxic situation can be introduced in between.

Under anaerobic conditions, transport and storage of simple organics such as acetates involve energy, from the polyphosphate mineral deposits of the microorganism with the discharge of phosphorus.

At the same time under successive aerobic period, the organic substance is oxidized to make energy and reconcentration of phosphates into polyphosphates.

The net effect is the excess of phosphorus in the bacterial cell .

A combination of bio-P removal with simultaneous chemical precipitation is likely to achieve low effluent phosphorus concentrations.

A flow-sheet for the process may be prepared as shown below:

METAL POLLUTION AND ITS BIOABATEMENT:

Metals and metalloids are ubiquitous in the biosphere. Rapid industrial developernent on the other hand, is gradually redistributing them. Though some of the metallic ions are essential for the continuation of life processes, yet others are known to be harmful at various concentrations to living organisms including man.

There is thus a great concern about their concentrations in the environment. This is particularly so with respect to the so-called 'heavy metals'. Although no specific definition has been attributed to these groups of metals, they are normally referred to as having a density higher than five.

Some of them in higher concentrations may even cause cancer. Cd has now been black-listed with Pb and Hg in this respect.

Heavy metals are considered to be a major field of interest by environmental scientists and engineers alike as they are increasingly polluting the air, water and soil environments.

Some of the most important and hazardous metals/ metalloids in this group are arsenic, cadmium, copper, chromium, mercury, lead, nickel and zinc.

They are highly toxic to the living systems and some might cause cancer in the long run even when injected in very low concentrations.

Toxicity is not only dependent on concentration and exposure, but is also known to increase with electropositivity of metals, viz. Hg > Cd > Zn.

Exhaustive studies throughout the world have not only yielded handsome knowledge of the physico-chemical properties of these toxicants, but also their biological impacts .

Differential solubility, mode of transport, interactions among different metal species, adsorption or absorption, chemical complexation with organic ligands, non-degradability, persistence in the environment, bio-accumulation, bio-magnification in the food chain and biomethylation are some of the characteristics which are worth consideraton.

Biomagnification is a phenomenon where the metal, absorbed by organisms at the lower trophic level of food chain, gradually get more concentrated in those organisms which are at the higher levels where man is the ultimate victim (At the highest tropic level of food chain man is there-so gradually concentrated metal ions enter human body-causing maximum damage).

Bio-methylation, on the other hand, involves the transfer of methyl group (CH3) from organic compounds to metals primarily due to the microbial activities in soil and water. Through this process some metallic ions get detoxified, yet others tum into more hazardous forms due to methyl conjugations.

Emission of toxic oxides into the air is the main source of heavy metal pollution. Emission of lead from automobiles accounts for two-thirds of global input.

While soils and water are considered as 'sinks' of toxic metals. In soils they, being immobile, accumulate in top soil and contaminate crops. In aquatic systems, the sources are industrial discharges, sewage, metal mining effluents, smelting, etc. Other sources include acid rains.

A voluminous literature have gathered with respect to the metal pollution menace and these need to be addressed suitably to tackle the problem effectively not only at the source level but also through abatement measures to minimize the impact.

The problem is rather acute in developing countries like India where resurgence of industrial activities is making a quantum jump in the process of economic upliftment and the threat of metal pollution is slowly becoming a reality.

Conventional physico-chemical techniques including precipitation, ionexchange, etc. were not only refined and stepped up, but efforts were also directed towards evolving suitable alternate biotechnological protocol.

Biosystem approach has not only helped in better understanding of the mechanism of metal toxicity and detoxification, and device effective mitigating measures, but has also opened up the possibility of utilizing these bioimpacts as pollution monitoring parameters.

Many of the aquatic plants, including phytoplanktons and benthics are gradually showing up their unique sorption potential of metals from the bathing media and thus act as natural bioscavengers of effluents.

Many microbes have also metal detoxification potential. Biosurveillance of toxic metals is still in the informative stage and is expected to turn out to be a real cost-effective (about 50- 70 per cent) device in the long-run.

This is inexpensive and effective too. Moreover, this approach would also help in the recovery of many industrially valued metals from further treatments of utilized biomass alongside the generation of biogas as energy source.

The recovery of metals, however, is facilitated when dead or immobilized biomass is used as adsorbent in the adsorption process rather than absorption.

CELL IMMOBILIZATION AS A TOOL IN WASTE TREATMENT:

Biotechnological processes, in general, involve the use of biocatalysts either in the form of specific enzymes or whole cell organisms. The same holds good not only in industrial processes, but also in the arena of environmental management.

Immobilization of such biocatalysts (enzymes/whole cells), particularly the microbial whole cell system, and possibility of their repeated use has opened up new avenues in pollution abatement.

Either the naturally occurring biodegrading microbes or their genetically improved counterparts (GEMs) can be maintained in substantially "unchanged" form to have continued expressions of biological activity and effectivity inside bioreactors through this novel technique.

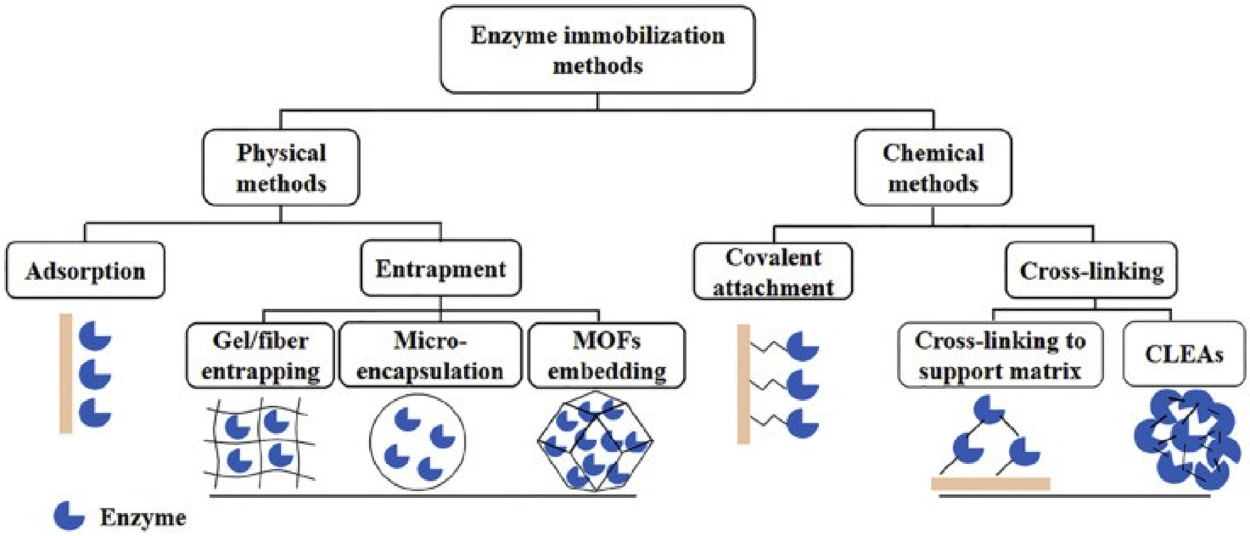
Advantages of Cell Immobilization

The immobilized cells offer considerable advantages over free cell suspensions, which lie in the

(i) Recovery and reuse,
(ii) Avoiding wash out of cells during operations,
(iii) Protection against extreme pH and temperature of the medium and toxic pollutants,
(iv) Possibility of easy separation from bathing solutions or reactors,
(v) Function of cellular multienzyme systems simultaneously to augment effectivity, where needed, against the use of single enzyme, and
(vi) To have a continuous process operation in biological reactors.

Techniques of Immobilization : Immobilization basically involves 'fixation' or confinement of cells or enzymes on to a support system. This can be achieved by entrapment and/or attachment.

Induced immobilization is effected through either physical or chemical means



Encapsulation may be effected with polyacrylamide while entrapment is done on a matrix of collagen or silicagel. The cells (living/dead) remain within a porous polymeric structure.

This is most widely used particularly in laboratories in case of both microbial and higher plant or animal cells from tissue cultures.

Alginate is a polysaccharide which when cross-linked by cations such as Ca", forms a gel and is highly effective in cell trapping.

However, it has low mechanical strength and is sometimes susceptible to biodegradation, whereas polyeurethanes are a class of nonbiodegradable polymers and serve as better cell immobilization agents.

Adsorption or Adhesion of cells on a carrier support is based on the concept that microbial cell surfaces are generally negatively charged and need the support which ought to be positively charged and A dense layer of cells or biofilm is formed and adhere on a solid support. Pretreatment of yeast, Arthrobacter cells with aluminium ions promotes adhesion on glass surface. .

Flocculation, on the other hand, helps aggregation of cells, as is usually done in activated sludge or fluidized-bed reactors. While cell to cell crosslinking is effected with polyelectrolytes or chitosans. This has been done with cells of Aspergillus, Lactobacillus and animal cells.

For proper development of reactors based on immobilized cells, knowledge of physiological state of the cells prior to, during and after immobilization should be known.

Some of the immobilized cell reactors (biological reactors) for waste treatments are trickling filter, basket reactor, rotating disc reactor and fluidized bed reactor.

Perhaps the first of this kind of reactors was made by Louis Pasteur, when he immobilized Acetobacter cells on the surface of woodchips by adhesion.

Environmental Applications of Immobilized Cells :

In environmental studies, immobilized cells have the greatest use in waste water treatments including recovery of metals from mine effluents.

Waste water containing various pollutants such as phenolics, cyanides, nitrates, ammonia, chlorinated compounds and even heavy metals can be suitably treated by this technique.

For phenol degradation, Pseudomonas putida cells were entrapped on sintered glass or activated carbon, while both P. putida and E. coli cells were entrapped in Ca-alginate to prevent damage to cell membrane during the treatment of 4-chlorophenol.

Ca-alginate immobilized Flavobacterium can degrade PCP (pentachlorophenol) in soil.

They have been used in the treatment of excessive fertilizer treated soils and fish hatcheries.

Domestic Waste Water Treatment

Conventional sewage treatment plants are based on biological decomposition of nontoxic organic wastes, using bacteria. Such biological decomposition conducted into aerobic conditions, i.e. in the presence of plenty of oxygen.

For oxidation of 1 mg of carbon, 2.67 mg of dissolved oxygen is required. Organic hydrogen, sulphur and nitrogen, the major elements in waste water, consume additional oxygen for their oxidation.

The solubility of O_2 in water is only 9 ppm (mg/L) at 20°C and less at higher temperature.

The purity of water depends on the rate of transport of O_2 by aeration and on the tota1 load organic material which requires oxidation.

The organic load is expressed in terms of BOD i.e. biochemical oxygen demand which means mg of O_2 needed to decompose the organic material in l L waste water

The processes commonly used for domestic sewage are discussed:

• In the first stage solid wastes are removed from water by screening and any scum is removed, followed by the settling of sludge.

• In the second stage, the residual liquid is subjected to biological of oxidation of soluble organic material through a bed of microbes in activated sludge. Finally, the solids are removed 'by sedimentation liquid effluent from such treatment has a much lower organic load after chlorination to kill pathogenic microorganisms, maybe mixed directly in lakes and streams In sewage treatment disposal

Primary treatment

• Primary treatment removes material that will either float or readily settle out by gravity. It includes the physical processes of screening, comminution, grit removal, and sedimentation. Screens are made of long, closely spaced, narrow metal bars.

• They block floating debris such as wood, rags, and other bulky objects that could clog pipes or pumps. • In modern plants the screens are cleaned mechanically, and the material is promptly disposed of by burial on the plant grounds. A comminutor may be used to grind and shred debris that passes through the screens. The shredded material is removed later by sedimentation or flotation processes.

Secondary treatment :

• Secondary treatment removes the soluble organic matter that escapes primary treatment. It also removes more of the suspended solids.

• Removal is usually accomplished by biological processes in which microbes consume the organic impurities as food, converting them into carbon dioxide, water, and energy for their own growth and reproduction.

• The sewage treatment plant provides suitable environment, albeit of steel and concrete, for this natural biological process.

• Removal of soluble organic matter at the treatment plant helps to protect the dissolved oxygen balance of a receiving stream, river, or lake.

• There are three basic biological treatment methods: the trickling filter, the activated sludge process, and the oxidation pond.

• A fourth, less common method is the rotating biological contacter.

• The effluents of secondary treatment plant is introduced into a flocculation tank where Ca(OR) (lime) is added to eliminate phosphates as Ca, (PO_4), (OH).

• From there the solution goes to NH_3, stripping tower.

• Nitrogen present in waste water exists as NH4 which is converted to gaseous NH; at high pH values (pH -11).

• The remaining organic materials are removed by adsorption on activated charcoal and finally chlorination is used for disinfection.

• The final effluent is fairly clean: BOD « 1ppm), PO4-0.2-1 ppm NH3; 0.3-1.3 ppm a N. However, tertiary treatment plants are costly due to their high energy requirements.

• The main energy costs are for recycling CaO obtained from lime sludge by heating and operating the NH_3stripping tower.

• An alternative method for nitrogen removal is transformation to N_2 using a combination of nitrifying and denitrifying bacteria.

• Oxidative degradation is accomplished mostly chemoheterotropic bacteria and ciliated protozoa.

• Effective degradation is caused by predominant genera like Pseudomonas, Zoogloea, Flavobacterium. Alcaligenes etc.

• Nitrification of waste is done by the action of the genre Nitrosomonas. Nitrospira.esc. in the first step and by Nitrobacter, Nitroscoccus etc.

• In the second step Denitrification takes place by the action of the genera Pseutiomonas, Microccus etc

Another-process is the lagooning process, the oldest process in the country. There are several types of this process (a) Aerobic lagooning, (b) Anaerobic lagooning (c) Aerobic-anaerobic lagooning.

Anaerobic Treatment Processes- In these processes about 95% of biodegradable C are decomposed into biogas (c.f. 50% in aerobic process) and the rest 5% into biomass.

• Three main steps are involved in the breakdown of organic waste under anaerobic conditions are:

• It is based on the settle ability of microbial floes to produce a region at the bottom of the digester where very high biomass concentration can be maintained.

• The effluent is fed in the bottom of UASB into the base of the sludge blanket. The size of the floes varies from 1 to 5 mm diameter and should have good settling characteristics.

• The loading rate is higher in comparison to other processes (19 kg. COD m3 -d -I). The UASB process satisfies the urgent need of developing countries for simple low cost and integrate environmental protection system, which combine waste water treatment with recovery and reuse.

Anaerobic waste water treatment is preferred to other processes for the reasons: i. Cheaper than aerobic process in respect of treatment of medium and high strength waste waters (COD 1500 mg L-I) . (ii) Energy (biogas) is produced instead of wasted. (iii) Less space is required for anaerobic plant (iv) Relatively low cost technology in terms of equipment UASB can be considered as the core of a very promising environmental protection and resource recovery concept.

Disposal of sludge • In sewage treatment disposal of sludge is one of the major problems The sludge can be spread on soil or landfills though the latter are becoming scarce with increasing population. • For localities near the shore the sludge is usually dumped into the ocean, which, however has a fragile ecosystem, in near future all kinds of ocean dumping should be stopped. • The Sludge may be utilised as fertilizer on crop lands. • There is the risk of contamination of-air with the toxic pollutants emitted from the sludge. • A safer procedure is an anarobic digestion sludge yielding CH4. • The effluent (liquid) from secondary treatment plants has a –lower BOD (25 mg/L). It has the composition: BOD 25 ppm, NH; 20ppm and PO~- 25Wm• For clear lakes this type of effluent will promote eutrophication.

Physical, Chemical and Biological Wastewater Treatment Methods The wastewater treatment is generally divided into three categories i. Treatment of water for domestic use ii. Treatment for specific industrial use iii. To treat wastewater to its acceptable for release into various water bodies or reuse Different treatment methods like physical, chemical and biological are used to treat the wastewater. General wastewater treatment consists of primary, secondary and tertiary wastewater treatments suspended, attached and hybrid reactors. Wastewater treatment options for the small communities are oxidation ditch, extended aeration system, SBR, aerated lagoon and waste stabilization pond system.

Sewage treatment plant: Domestic wastewater generated from wide range of activities and it contains oil, greases, oxygen demanding materials, sediments, pathogenic bacteria, viruses, pesticides, heavy metals and refractory organic compounds. Treatment of sewage wastewater is divided into physical, chemical and biological methods. Below table describes the various conventional physical, chemical and biological treatment options.

Physical Treatment	Chemical Treatment	Biological Treatment	Advanced treatment methods
Sedimentation Screening Aeration Filtration Flotation Degasification Equalization	Chlorination Ozonation Neutralization Coagulation Adsorption Ion Exchange	Aerobic treatment: Activated sludge treatment Tricking filtration Oxidation ponds Lagoon Aerobic digestion Anaerobic treatment : Anaerobic digestion Septic tank Lagoons	Activated carbon adsorption Membrane filtration Ion Exchange

Primary treatment:

In this method flow equalization, grit removal, grease and oil followed by primary sedimentation are carried out to remove large objects and settable matters in the wastewater. Primary treatment is a screening process mainly employed to remove the insoluble organic materials like floating grit, oil, grease and scum. In this process, large particle are removed initially followed by large particle are reduced to the level in which particle enter into sewage flow. Grit removal is used to prevent the accumulation in other parts of the treatment system which helps in controlling clogging of pipe and other parts.

Primary Sedimentation In primary sedimentation the settleable and floatable solids are removed by using flocculants. The floating materials in the primary settling tank called as grease. Greases consist of waxes, oils and free fatty acids. Generally the grease settles with sludge and surface floats are removed through skimmers. Settling Tank in which water remain for a long time in which suspended particles are settled down this process is known as sedimentation and it can be accelerated by adding chemical substance known as coagulant like alum.

Secondary Waste Treatment: Secondary treatment is designed to remove biodegradable organic matter in wastewater. In this biological process bacteria and protozoa consume biodegradable soluble organic contaminants like sugars, short chain-organic molecules and fats. In this process, microorganisms provided with substantial oxygen degrading organic matters to reach the BOD level to acceptable level. Optimum environmental condition is very important in this biodegradation process where bacterial growth is influenced. Secondary treatment systems are classified as fixed-film or suspended-growth system such as trickling filters, constructed wetlands, bio-towers, rotating biological, moving bed biofilm reactors (MBBR), integrated fixed-film activated sludge (IFAS) processes and activated sludge.

Trickling filter: It is one of the simplest aerobic biological treatment methods. In this technique, wastewater is sprayed fixed bed such as rock, gravel, slag, polyurethane foam, sphagnum peat moss and ceramic covered with millimeters thick gelatinous matrix (species of bacteria, cilliates and amoeboid protozoa).

Rotating biological reactors (contactors): This is another type of biological treatment method. In this method large plastic discs mounted with rotating shaft in contact with air. Each rotating disc made of high-density polyethylene or polystyrene in which thin layers of attached biomass is grown. The rotating biomass containing disc degrades organic matter in the sewage. Rotating biological reactors and trickling filters are working in the principle of attached growth processes called fixed-film biological (FFB). Due to advantage of this process is low energy consumption, trickling filter is used in most of the wastewater treatment plants.

Activated sludge process: One of the most flexible and effective process applied in wastewater treatment processes. In this method microorganism utilize the wastewater containing organic contaminates in to microbial biomass and CO_2. The microorganism and wastewater is stirred in large quantities to provide oxygen demand of microorganisms and keep solids in suspension. Activated sludge process effectively removes more than 95% BOD reduction in wastewater. The microbial strain in the degradation processes is generally maintained until it complete the log phase of growth in the aeration tank and further cells settle as solids. The settled particles are removed in settling tank and part of solid part recycled the head of aeration tank as a seed microbial inoculum comes into contact with fresh wastewater. Rich organic load and adopted microbial inoculum in return sludge helps in effective degradation of organic matter.

Activated sludge process :

In this method, BOD is removed by oxidation of organic matter and generation energy for the metabolism of the microorganisms in which carbon is converted in to CO_2. In second pathway the synthesis and incorporation of the organic matter into cell mass. The waste sludge generated from the process create disposal problem due to its undesirable constituents. Some extent the sludge disposed in landfill or used as fertilizer to condition the soil. However, before application in soil, it needs to be analyzed for excessive heavy metals.

Constructed wetlands Constructed wetlands are artificial wetland mainly applied to treat the municipal, industrial wastewater and grey water or stormwater runoff. This is like a bio-filtration system in which various aquatic plants are used to remove the contaminants. Generally constructed wetlands are designed with 18 feet shallow deep pit lined with thick plastic liners filled with planted with evenly spaced wetland plants. In this method the sediments and pollutants like heavy metals are removed effectively. This treatment system is relatively inexpensive and easy to construct, maintain and operate. Also ecologically sound method and used to treat medium and small volume of wastewater.

Anaerobic treatment Anaerobic digestion process is a serious of biological process by action of microorganism under anaerobic condition. In this method anaerobic bacteria convert the organic waste materials into methane. Various conventional and high rate anaerobic digesters are used to municipal wastewater solids, high strength industrial wastewater and residuals, fats, oils and grease and various other organic waste streams into biogas.

Tertiary and Advanced Wastewater Treatment:

Tertiary treatment process also called as advanced treatment system used to remove specific organic and inorganic constituents from industrial effluent to make it suitablefor reuse. In this process treated wastewater suitable for land application purpose or directly discharge it into the water bodies like rivers and lakes. The target contaminants like suspended solids, dissolved organic and dissolved inorganic materials are treated. The tertiary wastewater treatment methods include membrane filtration, ion exchange and activated carbon adsorption.

Membrane process:

Membrane treatment technology plays a vital role in wastewater treatment and become a increasing important. It mainly used to remove particles, colloids and micrmolecules. In this process separation takes place without phase change. There is no accumulation of product inside membrane and there is no additive required for separation of pollutants. The membrane methods are electrodialysis, microfiltration, ultrafiltration, nanofiltration and reverse osmosis. Electrodialysis works with direct current to separate into vertical layer by membranes permeable to cations and anions. Cations migrate towards the cathode and anions move towards anode. Reverse Osmosis is very important and well developed technology for purification of water. In this process water forcing to enter through semipermeable membrane to allows the passage of water and not for the pollutants.

Membrane bioreactor It is combination of a membrane process like microfiltration with a biological wastewater treatment process like activated sludge process. It provides an efficient organic and suspended solids removal. In this method membranes used with size of 0.035 microns to 0.4 microns submerged in an aerated biological reactor. This method replaces the need of sedimentation and filtration processes in the wastewater treatment and allows high quality effluents. Various parameters affect the performance of membrane bioreactors such as membrane characteristics, sludge characteristics and operation conditions.

ACTIVATED SLUDGE:

Activated sludge is a process that uses a biological system to treat sewage or industrial wastewater.

• In this process, the microbial consortium reduces the organic load of the wastewater converting the organic matter into microbial biomass and Carbon di oxide.

• During growth and as well by aeration process the microbial biomass clump together and occur as aggregates or flocs and is called as Activated Sludge.

Process The process of activated sludge involves the following steps

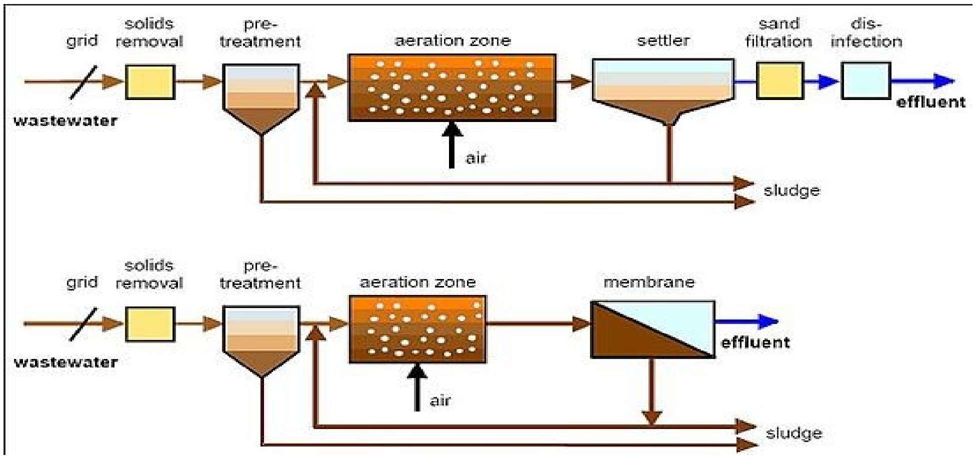
1. Aeration of wastewater Aeration provides oxygen and accomplishes mixing of wastewater and microorganisms. Microorganisms utilize oxygen for growth.
2. • Diffused or surface mechanical aeration is used as the aeration source.
3. • Efficient operation of activated sludge process requires a constant supply of oxygen. Single or multiple aeration tanks are used depending upon the type of activated sludge process. Mixture of microbes or activated sludge and wastewater is called mixed liquor.
   Solid liquid separation In the settling tank, the mixed liquor solid is separated from the treated effluent or wastewater. The biomass as flocculent sludge settles down.
   • Several secondary clarifiers are also employed in some treatment plant operations

4. Discharge of treated effluent The treated effluent is discharged from the secondary clarifier as clear water

   4. Return of settled sludge to aeration tank A part of the solids settled in the clarifier is returned to the aeration tank to maintain the concentration of the microorganisms. This portion of floc is called as return sludge

5. Disposal of remaining activated sludge Excess activated sludge is sent to disposal or sludge handling system to maintain the system at a steady state Disposal of excess sludge is to uphold appropriate concentration of microorganisms in the aeration system and to efficiently degrade the BOD Excess microorganisms removed from the activated sludge system are called as Waste Activated sludge (WAS)
6. A balance is always maintained between the growth of new organisms and their removal by wasting. Wastage of more sludge decreases the concentration of microorganisms in the mixed

liquor that will ultimately affect the treatment process. Low removal of sludge results in large concentration of microorganisms which will reach the secondary tank and reaches the receiving stream.



Advantages of activated sludge :

Removes suspended solids

- Nitrification, denitrification and phosphorus removal
- Solids and liquids separation
- Removes organics
- Effective in removing pathogens

Disadvantages of activated sludge:

Process quality gets affected by change in volume of sewage or sewage characteristics High operation cost

- Large scale sludge disposal
- Skilled supervision required to check that the returned sludge remains active

OXIDATION DITCH: An oxidation ditch is a modified activated sludge biological treatment process that uses long solids retention times (SRTs) to remove biodegradable organics.

• This treatment system consists of an elliptical or ring shaped basin. Aerators are mounted horizontally or vertically in the basin

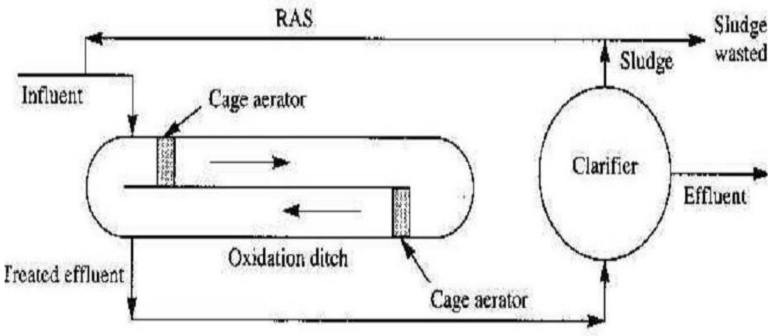
• The aeration equipment called the aeration rotor provides aeration as well circulates the wastewater or the effluent and this allows the waste water to have plenty of exposure to the open air for the diffusion of oxygen.

• The dissolved oxygen in the wastewater increase and gradually decreases as microorganisms take it for biomass production

• The oxidation ditch effluent is clarified in a separate secondary clarifier and the settled sludge is returned to maintain a desirable MLSS concentration.

• The MLSS concentration in the oxidation ditch generally ranges from 3,000 mg/ L to 5,000 mg/ L

• The MLSS concentration in the oxidation ditch depends upon the surface area provided for sedimentation, the rate of sludge return, and the aeration process.
• Longer retention time within the ditch will allow for a greater amount of organic matter to be broken down by the aerobic bacteria.
• After treatment, the waste water is pumped to a secondary settling tank where the sludge and the water are allowed to separate.
• From there the effluent goes on to other treatment processes or disposal.
• Pasveer and Carrousel types of oxidation ditches are extended aeration systems



Typical layout of an oxidation ditch system.

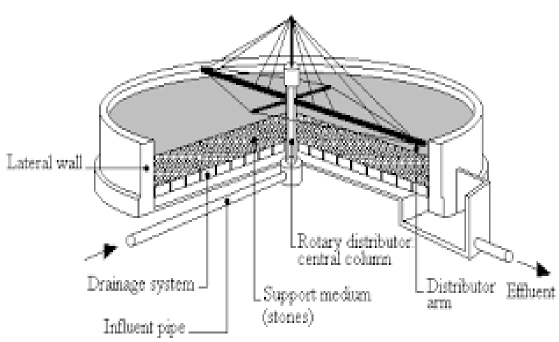
Advantages :
 Withstands high shock load or hydraulic surge.
- Moderate operating cost
- Reliable technology over other biological processes.
- Highly effective compared to other process
- Less sludge production compared with other biological treatments as the process has
- an extended biological activity Energy efficient process
- Disadvantages Construction and working requires large area of land.
- Requires high capital cost
- High concentration of suspended solids

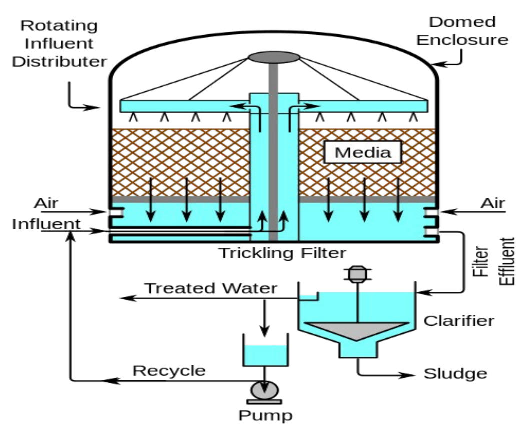
TRICKLING FILTER:
Its an aerobic biological process for sewage and waste water treatment
- Also called as biological trickling filter
- This system utilizes microorganisms attached to the material such as stones, slats or plastic to remove or degrade organic matterProcess Trickling filter consists of a cylindrical tank and contains a bed of coarse materials
- such as rocks, slats, gravel, PVC bottles or preformed plastic material that provide a high surface area The wastewater is trickled over the surface of the sand, gravel or any packing material

l• Rocks provide surface area for biofilm formation
• When wastewater is trickled, microorganisms come in contact with the organics and metabolize it. This results in slime layer thickness. The biological film is rich in the bacteriaPseudomonas, Flavobacterium, Alcaligenes, and algae-Chlorella, Ulothrix, and Stigeoclonium, besides some fungi and yeasts. Biofilms with a thickness in the range of 70 -100 pm are efficient for the treatment process. Oxygen penetration to the medium is slowed because of excess thickness of biofilm formation. Anaerobic organisms start developing
• Increase in biofilm growth, makes the microorganisms sloughs from the media surface, thereby a portion of biofilm falls and is washed out of the bed by the wastewater
• These solids from the filter sediments in a sedimentation tank called the final clarifier.
Classification of trickling filters
• Classification of trickling filters is mainly based upon the hydraulic and organic load
• Hydraulic load – Expressed in cubic meters of wastewater applied per day per square meter of bulk filter surface area or depth of water applied per unit time (m/d)
• Organic loading - Expressed as Kilograms of 5 day 20∘C BOD per day per cubic meter of bulk filter volume (kg/d.m3)
• Recirculation – In trickling filter, provision is provided for return of a portion of the effluent to flow throught the filter called as recirculation. – Recirculation ratio is the returned flow to the incoming flow ratio
• Based upon the hydraulic and organic load, the trickling filter is classified in to Low rate filters• Intermediate rate filters

- High rate filters
- Super rate filters
- Roughing filters

Roughing filters are a special type of trickling filters that operate at high hydraulic Ioading rates. These reduce the organic matter in downstream processing.





Advantages: Low operating cost

- Sludge removal is easy
- Low power requirements

- Efficient in removal of ammonia
- Produce less sludge than suspended growth systems

Disadvantages Treated effluent should be treated additionally to meet the discharge standards

- Odour and vector problem
- High incidence of Clogging