ENVIRONMENTAL BIOTECHNOLOGY

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INTRODUCTION

- ❖ The ***environment*** is a very important component necessary for the existence of both man and other biotic organisms. The ***degree of sustainability of the environment is an index of the survival and well-being of the entire components in it.***
- ❖ The global environment is now facing a highly critical situation due to rapid urbanization and industrialization as well as increasing population and others in the limited natural resources.
- ▪ The economic growth reflects the drastic changes of the life style of the people that created anthropogenic stress on the environment & create environmental problems such as pollution.
- ▪ *Environmental pollutants are now the major global concern due their undesirable recalcitrant and xenobiotic compounds*.
- ▪ E.g.: A variety of polycyclic aromatic hydrocarbons (PAHs), chlorinated and nitro-aromatic compounds, heavy metals and xenobiotics, were depicted to be highly toxic, mutagenic and carcinogenic for all living organisms in the earth.
- ❖ As a result, there is requirement of highly developed environmental management systems and search of biotechnological technology ***to reduce and remove the contaminated materials and reestablish and restore the ecosystem and improved utilization of natural resources***.
- ❖ Therefore, number of MOs and plants are considered to be the best suitable candidates among all living organisms to remediate most of the environmental contaminants into the natural biogeochemical cycle due to their ***diversity, versatility and adaptability in adverse conditions***.
- ▪ These MOs & plants exhibit a remarkable range of contaminant degradable capacity that can proficiently restore the natural environmental conditions.
- ➢ Now with recent advancement of the *genetic approach* multiplies the bioremediation process for protection of the natural environment by recycling the waste materials.

❖ The development of multiple human activities (in industry, transport, agriculture, domestic space), the increase in the standard of living and higher consumer demand have amplified ***pollution of air*** (with CO2, NOx ,SO2, greenhouse gasses, particulate matters), ***water*** (with chemical and biological pollutants, nutrients, leachate, oil spills), ***soil*** (due to the disposal of hazardous waste, spreading of pesticides), ***the use of disposable goods or non-biodegradable materials, and the lack of proper facilities for waste handling and treatment*** **(Fig. 1).**

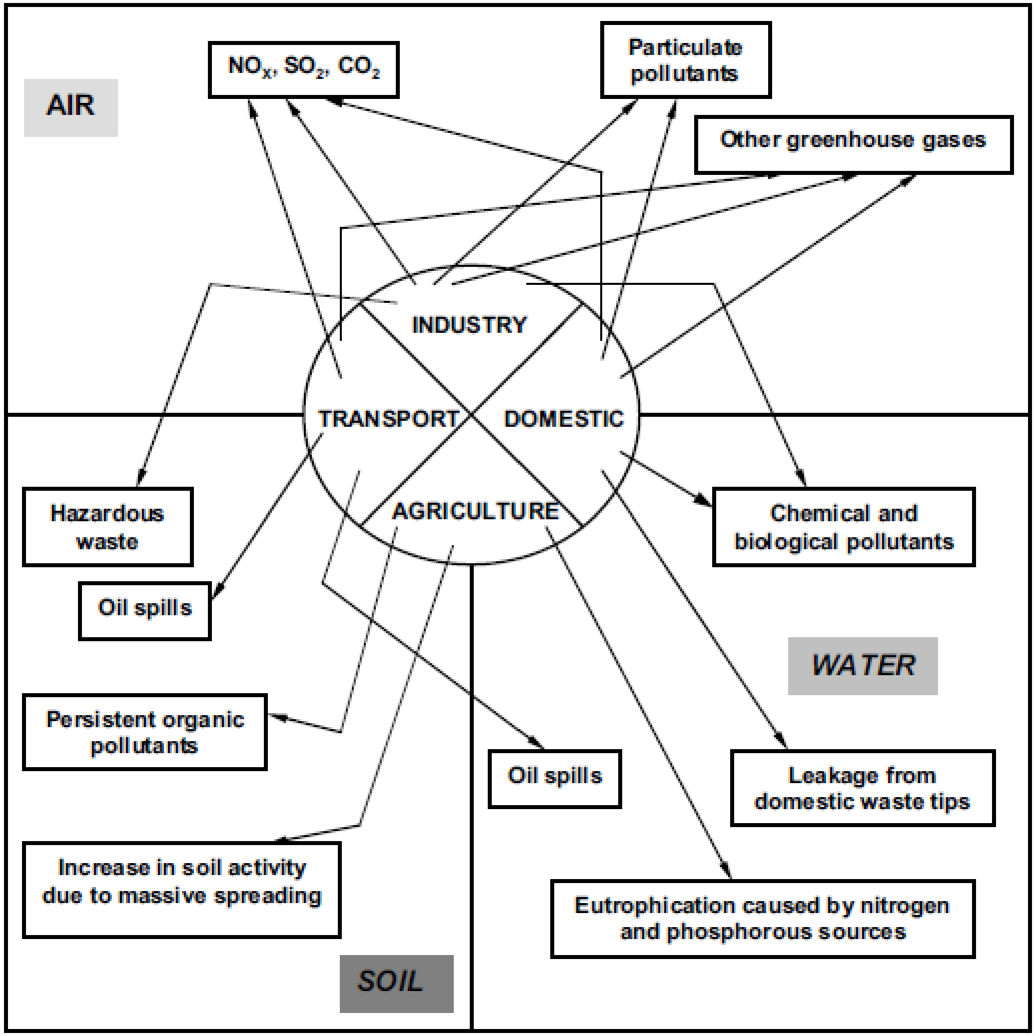


Figure 1. The spider of environmental pollution due to anthropogenic activities. (Adapted from EIBE 2000).

❖ Studies and researches demonstrated that some of these pollutants can be ***readily degraded or removed by biotechnological solutions***, which involve the ***action of microbes, plants, and animals*** under ***certain conditions*** that ***envisage abiotic and biotic factors***, ***leading to non-aggressive products through compounds mineralization, transformation or immobilization*** **(Fig. 2).**

Sources of pollutants and factors affect the removal

- Enormous quantities of pollutants have been released into the environment.
  - Those ***structurally related to natural compounds are readily degraded or removed by microorganisms as well as plants*** (Phytoremedation) and ***animals*** found in soil and water.
  - However, superimposed on the wide variety of pollutants present in the environment is an ***increasing number of novel industrial compounds rarely found in nature.***
- These ***xenobiotic and heavy metals compounds*** are usually removed slowly and tend to accumulate in the environment. Due to the high degree of toxicity, their accumulation can cause severe environmental problems.(Fig 2).

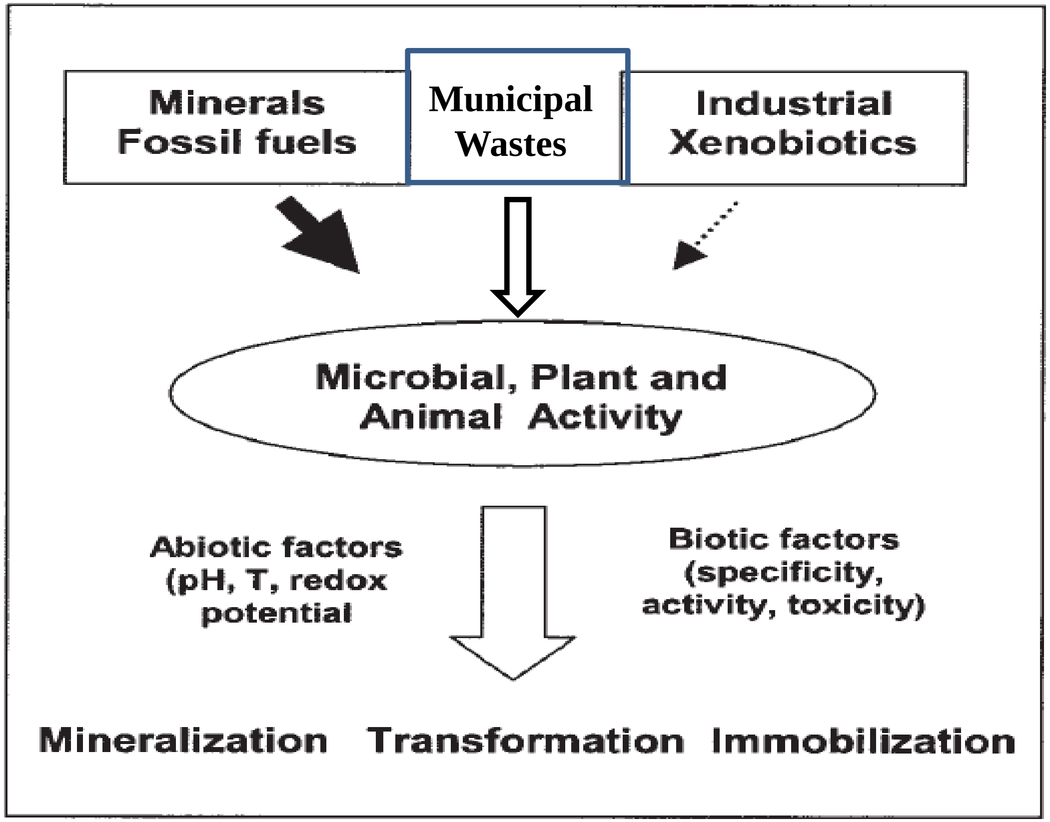


Figure 2. Main sources of environmental pollutants and factors influencing their nature removal from the environment (Adapted from Chen et al 2005).

- Some contaminants have been shown to be uncommonly **recalcitrant**, i.e. *microorganisms neither metabolize nor transform* them into certain other nontoxic metabolites.
- As a result, it may be more productive to discover new catabolic pathways that might lead towards complete mineralization of these toxic pollutants.

❖ Advanced techniques or technologies are now possible to treat waste and degrade pollutants ***assisted by living organisms*** or ***to develop products and processes that generate less waste and preserve the natural non-renewable resources and energy*** as a result of:

- improved treatments for solid waste and wastewater;
- bioremediation: cleaning up contamination and phytoremediation;
- ensuring the health of the environment through biomonitoring;
- cleaner production: manufacturing with less pollution or less raw materials;
- energy from biomass;
- genetic engineering for environmental protection and control.

❖ Unfortunately, some environmental contaminants are refractory with a certain degree of toxicity and can accumulate in the environment.

▪ Furthermore, the treatment of some pollutants by ***conventional methods***, such as chemical degradation, incineration or landfilling, ***can generate other contaminants***, which superimposed on the large variety of noxious waste present in the environment and ***determine increasing consideration to be placed on the development of combination with alternative, economical and reliable biological treatments.***

❖ ***At least four key points are considered for environmental biotechnology interventions*** to **detect** (using biosensors and biomonitoring), **prevent in the manufacturing process** (by substitution of traditional processes, single process steps and products with the use of modern bio- and gene technology in various industries: food, pharmaceutical, textiles, production of diagnostic products and textiles), **control and remediate the emission of pollutants** into the environment (**Fig. 3**) (by degradation of harmful substances during water/wastewater treatment, soil decontamination, treatment and management of solid waste).

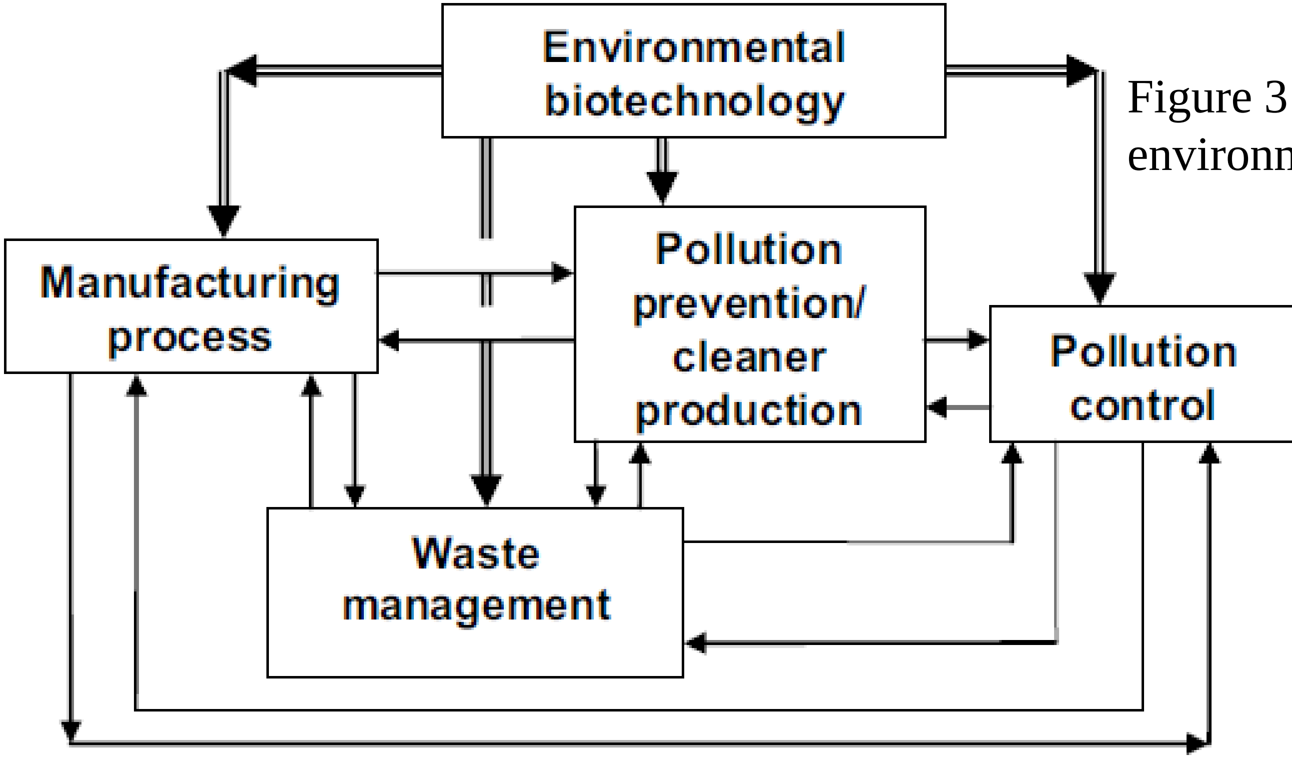


Figure 3 Key intervention points of environmental biotechnology.

- ❖ Other significant areas where environmental biotechnology can contribute to pollution reduction are production of biomolecules (proteins, fats, carbohydrates, lipids, vitamins, aminoacids), yield improvement in original plant products.
- ▪ The production processes themselves can assist in the reduction of waste and minimization of pollution within the so-called clean technologies based on biotechnological issues involved in reuse or recycle waste streams, generate energy sources, or produce new, viable products.
- ➢ ***By considering all these issues, biotechnology may be regarded as a driving force for integrated environmental protection by environmental bioremediation, waste minimization, environmental biomonitoring and biomaintenance.***

- **Scientists learn from nature in the 1980's**
  - ***The concept of Gaia*** –the total world is a living organism and what nature makes nature can degrade (bioinfalibility); i.e, only man makes xenobiotic compounds.
    - Clean up pollution-short and long term solutions (cost, toxicity, time frame)
    - Use compounds that are biodegradable
    - Produce energy and materials in less destructive ways
    - Monitor environmental health
    - Increase recovery of minerals and oil

- ***What do they all have in common?***
    - increase in products and waste
    - people moved to the city
    - increase in human population

- ***What are the events that triggered the interest in environmental biotechnology?***
  - Rachel Carlson's Silent Spring (DDT)
  - Love Canal
  - Burning of a River
  - Exxon *Valdez* oil spill in 1989

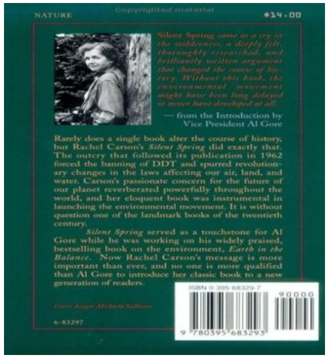


Fig.4. Environmental pollution due to human activities

❖***What Needs to be Cleaned UP?***

- ***How remediation is used depends on***

  1) what is contaminated? (solid, liquid or gas and locations)
  2) the types of chemicals that need to be cleaned up
  3) the concentration of the contaminants and period (amount and duration)

❖***Bioremediation finds its place***

- Companies begin to specialize in cleaning up toxic waste spills by using a mixture of bacteria and fungi because cleaning these spills usually requires the combined efforts of several strains. (The use of microorganisms to remedy environmental problems.)
- Biotechnologists begin engineering "super bugs" to clean up wastes using many microorganisms in nature that will degrade waste products.

❖ Environmental hazards and risks that occur as a result of *accumulated toxic chemicals or other waste and pollutants could be reduced or eliminated through the application of biotechnology in the form of (bio) treatment/(bio) remediating* historic pollution as well as addressing pollution resulting from current industrial practices through pollution prevention and control practices.

❖ ***Bioremediation*** is defined by US Environmental Protection Agency (USEPA) as "a managed or spontaneous practice in which microbiological processes are used to degrade or transform contaminants to less toxic or nontoxic forms, thereby remediating or eliminating environmental contamination" (USEPA 1994; Talley 2005).

- ***Biotreatment /bioremediation*** methods are almost typical **"end-of-pipe processes"** applied to remove, degrade or detoxify pollution in environmental media including water, air, soil and solid waste.

❖ ***Four processes can be considered as acting on the contaminant:***

1. ***Removal:*** a process that physically removes the contaminant or contaminated medium from the site without the need for separation from the host medium;
2. ***Separation:*** a process that removes the contaminant from the host medium (soil or water);
3. ***Destruction/degradation:*** a process that chemically or biologically destroys or neutralizes the contaminant to produce less toxic compounds;
4. ***Containment /immobilization:*** a process that impedes or immobilizes the surface and subsurface migration of the contaminant;

❖ ***Removal of any pollutant from environment use Two routes:***

- ***Removal, separation, and destruction/degradation*** are processes that reduce the concentration or remove the contaminant and results unavailable for degradation.
- ***Containment/immobilization*** on the other hand, controls the migration of a contaminant to sensitive receptors without reducing or removing the contaminant.

❖ ***Bioremediation –***

- Use microbes into the ground to clean up or deactivate groundwater pollution.
- This process, called ***bioremediation*****,** modifies bacteria and fungi that naturally break down toxins so can clean up chemical spills, waste dumps and even radioactive waste sites faster d and more efficiently than without their help.

❖ Though bioremediation approach has been used at varying degrees for more than 60 years, like petroleum land farming, it has been implemented as a very 'black box' engineering solution where amendments are added and the pollutants are degraded (Chakraborty et al., 2012).

❑ A summary of processes involved in bioremediation as a generic process is presented in Fig. 5.

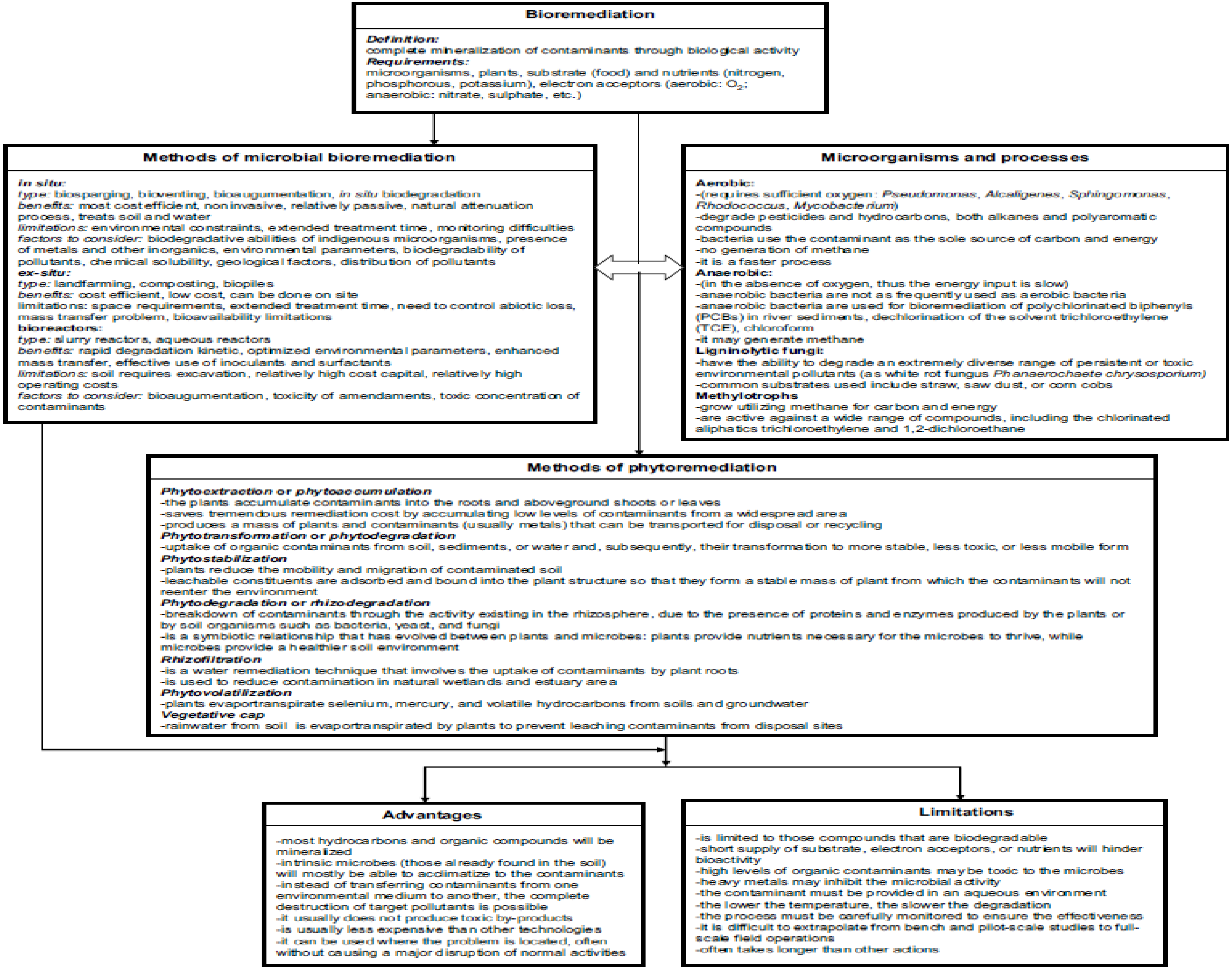


Figure 5 Characteristics and particularities of bioremediation. (Adapted from Vidali 2001; Gavrilescu 2004a).

❖ ***The two routes for removal of contaminants:***

A) Destruction (biodegradation and biotransformation) is carried out by an organism or a combination of organisms (consortia) and is the core of environmental biotechnology, since it forms the major part of applied processes for environmental cleanup.

- ***Biotransformation processes*** use natural and recombinant microorganisms (yeasts, fungi, bacteria), enzymes and whole cells, and plays a key role in the area of waste treatments, foodstuff, pharmaceutical industry, vitamins, specialty chemicals and animal feed stock.

B) Immobilization can be carried out by chemicals released by organisms or added in the adjoining environment, which catch or chelate the contaminant, making it insoluble, thus unavailable in the environment as an entity. Sometimes, immobilization can be a major problem in remediation because it can lead to aged contamination and a lot of research effort needs to be applied to find methods to turn over the process.

➢ ***Biological processes*** rely on useful microbial reactions including degradation and detoxification of hazardous organics, inorganic nutrients, metal transformations, applied to gaseous, aqueous and solid waste.

- A ***complete biodegradation*** results in detoxification by mineralizing pollutants to carbon dioxide, water and harmless inorganic salts.
- ***Incomplete biodegradation*** will yield breakdown products which may or may not be less toxic than the original pollutant and combined alternatives have to be considered, such as: dispersion, dilution, biosorption, volatilization and/ or the chemical or biochemical stabilization of contaminants.

❖ In addition, ***bioaugmentation*** involves the deliberate addition of microorganisms that have been cultured, adapted, and enhanced for specific contaminants and conditions at the site.

- ***Biorefining*** entails the use of microbes in mineral processing systems and, in some cases, enables the recovery of minerals and use of resources that otherwise would not be possible.
- The key to ***successful bioremediation*** is *to trap up the naturally occurring* ***catabolic potential*** *of microorganisms to effectively catalyze transformations* of environmental pollutants.
- Small scale experiments using distinct microbial consortia in the laboratory is an immense starting point in providing crucial initial indication of the bioremediation process within definite control condition.

- ***However bioremediation in real execution*** is a *complex phenomenon involving more than one contaminant and simultaneously mediated by different microorganisms involving different metabolic pathways*, *across geochemical gradients, geophysical and hydrological complexities* (Purohit, 2003).
- Thus, in addition to consider type, concentration and duration of pollutants as well as selection of microbes and exposure time, it is crucial to search for alternative advanced technology for efficient, cost effective and environmental friendly treatment methods.

Reasons for Searching Alternative Advanced Technology for Treatment

- Because of the problems associated with conventional treatment methods of wastes, such as incineration, landfills or chemical, increasing consideration should be placed on the development of alternative, economical and reliable integrated treatments + biologically.
- Natural microorganisms evolution occurs at a relatively slow rate, particularly when the acquisition of multiple catalytic activities is necessary.
- The acceleration of resistant microbes via genetic engineering/biochemical engineering is helpful since the desirable traits can be carefully designed and controlled.
- The drive toward this goal represents the essence of **environmental biotechnology**.

❖ What is Biotechnology?

- The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services.
- Use and applications of ***biological system*** (cells, tissues, etc), ***biomolecules*** (enzymes/proteins, antibodies, DNA/RNA) and ***key technologies*** to produce valuable products at commercial scale and to treat diseases and polluted environments.
- The integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services.
- The living organisms in definition include microorganisms, enzymes, cells of animal and plant.

➢ ***Biotechnology*** *is defined as "fermentation using bioreactors, bioprocessing, bioleaching, biopulping, biobleaching, biodesulphurisation, bioremediation, biofiltration and phytoremediation" (OECD, 2005).*

❖**Biotechnology** is versatile and has been assessed a key area which has greatly impacted various technologies based on the application of biological processes in manufacturing, agriculture, food processing, medicine, environmental protection resource conservation (Fig.6).

❖ This new wave of technological changes has determined dramatic improvements in various sectors since it can provide entirely novel opportunities for sustainable production of existing and new products and services.

➢ In addition, ***environmental concerns*** help drive the use of **EB** not only for pollution control (decontamination of water, air, soil), but prevent pollution and minimize waste in the first place as well as for environmentally friendly production of chemicals and biomonitoring.

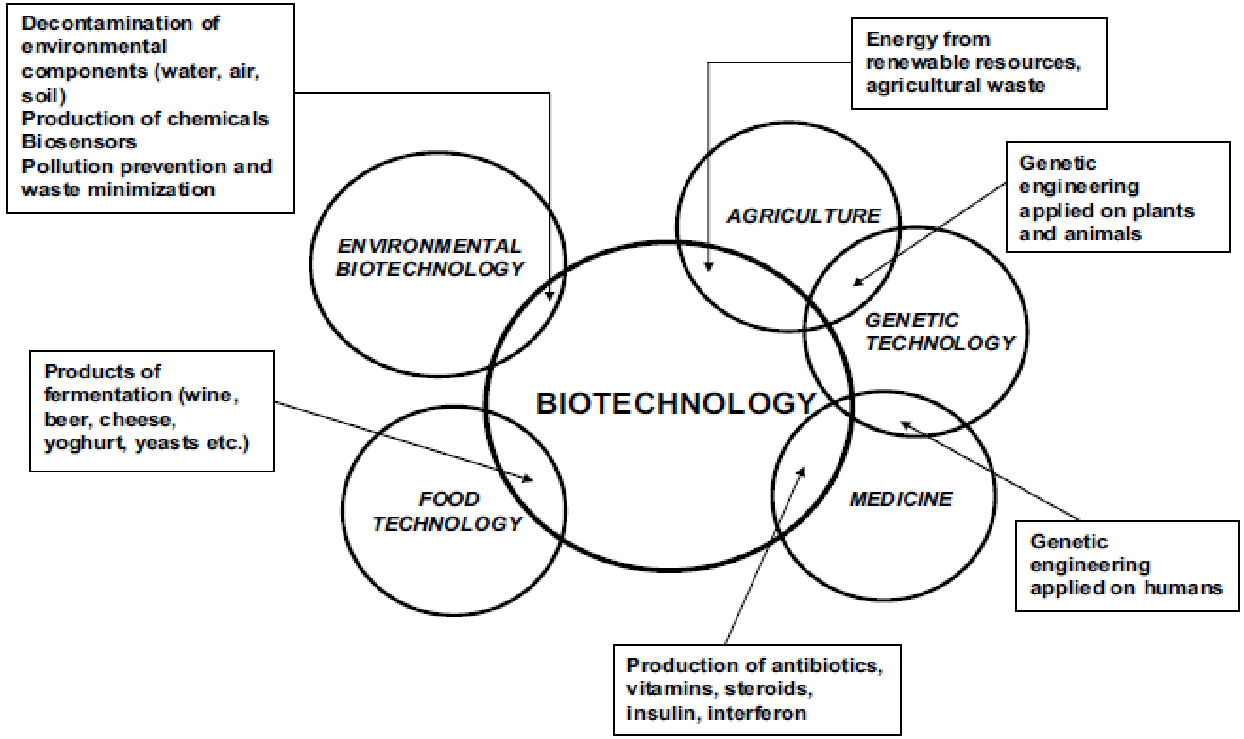


Figure 6 Application of Biotechnology in anthropogenic activities (industry, agriculture, medicine, health, environment) (Adapted from Sukumaran Nair 2006).

- **The responsible use of biotechnology** ***to get economic, social and environmental benefits*** is inherently attractive and determines a spectacular evolution of research from traditional fermentation technologies, to modern techniques (gene technology, recombinant DNA technologies, biochemistry, immunology, molecular and cellular biology) to provide efficient synthesis of low toxicity products, renewable bioenergy and yielding new methods for environmental monitoring.
- The main action areas for biotechnology as important in research and development activities can be seen as falling into **four** main categories:

  - ***industrial supplies*** (biochemicals, enzymes and reagents for industrial and food processing);
  - ***agriculture*** and ***energy*** (biofertilizer, biopesticides; fuels from renewable resources);
  - ***environnent*** (pollution diagnostics, products for pollution prevention, bioremediation).

- These are successfully assisted by various disciplines, such as *biochemical bioprocesses, biotechnology engineering, genetic engineering, protein engineering, metabolic engineering,* required for commercial production of biotechnology products and delivery of its services.

- As a recognition of the strategic value of biotechnology, integrated plans are formulating and implementing in many countries for using biotechnology for industrial regeneration, job creation and social progress.
- With the implementation of legislation for environmental protection in a number of countries together with setting of standards for industry and enforcements of compliance, *environmental biotechnology* gained in importance and broadness in the 1980s.

- *Regarding these domains of application, **four main subfields of biotechnology** are usually described about:*

- ***Green biotechnology***, the oldest use of biotechnology by humans, deals with plants and growing;
- ***Red biotechnology***, applied to create chemical compounds for medical use or to help the body in fighting diseases or illnesses;
- ***White biotechnology*** (often green biotech), focusing on using biological organisms to produce or manipulate products in a beneficial way for the industry;
- ***Blue biotechnology*** – aquatic use of biological technology.

- Generally, **biotechnology** can be classified in to five broad areas:

a) **Environmental Biotechnology (EB)**
b) Industrial biotechnology
c) Agricultural biotechnology
d) Health biotechnology
e) Microbial biotechnology

- The social, environmental and economic benefits of ***environmental biotechnology*** go hand-in-hand to contribute to the development of a more sustainable society a principle which was promoted in the Brundtland Report in 1987, in Agenda 21 of the Earth Summit in Rio de Janeiro in 1992, the Report of the World Summit on Sustainable Development held in Johannesburg in 2002 and which has been widely accepted in the environmental policies.

EB: the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues ***for the protection and restoration of the quality of our environment.***

✓**EB** is concerned with the application of biotechnology as an emerging technology in the context of environmental protection, since rapid industrialization, urbanization and other developments have resulted in a threatened clean environment and depleted natural resources.

Environmental Biotechnology

- The distinct role of **EB** in the **future** is emphasized considering the opportunities to contribute with new solutions and directions in remediation of contaminated environments, minimizing future waste release and creating pollution prevention alternatives.

- Evolved from the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for the protection and restoration of the quality of our environment.

- The development, use and regulation of biological systems and engineering for remediation of contaminated environments (land, air, water), and for environment-friendly processes.

➢ Offers the most economical and environmentally effective method for solid, liquid and gasses/air pollution control when dealing with the removal of odorous and toxic contaminants from industrial and municipal waste disposal sites and airstreams.

❖ **Environmental Biotechnologies provide valuable services to society:**

a) Treating industrial and municipal wastes to protect water and land resources, ecosystems, and human health through restoring sites contaminated with hazardous materials;

b) Reclaiming impaired water resources and soils;

c) Capturing renewable resources, particularly energy and biofertilizers; and

d) Producing environmentally benign products.

Environmental Biotechnology from Chemical Engineering Perspectives

- In its early stage, environmental biotechnology has evolved from chemical engineering, but later, other disciplines (biochemistry, environmental engineering, environmental microbiology, molecular biology, ecology) also contribute to environmental biotechnology development.
- **EB** refers to the utilization of microorganisms, plants and animals in combination with chemical engineering to improve environmental quality.
- Starting with the use of activated sludge and anaerobic digestion in the early 20th century by civil engineers, the introduction of new technologies from modern microbiology and molecular biology in to chemical engineering has enabled engineers and scientists to tackle the more contemporary environmental problems such as detoxification of hazardous chemicals.
- ***Chemical engineers*** are uniquely poised to contribute in this emerging area since many of the ***potential solutions*** **require a combined perspective** from modern biotechnology, process, biochemical and environmental engineering, four areas where chemical engineers excel.
- For example, the realization of environmental biotechnology into practical solutions requires the implementation of process design and synergetic treatment methods, which is the foundation of the chemical engineering discipline.
- ***Modern chemical engineering***, a deeper inspection reveals that the design of biological catalysts, based on defined techniques from ***biochemistry and biology*** is indeed parallel to our understanding of ***chemical kinetics, transport, separation and control phenomena***.
- Even though chemical engineers are well prepared to contribute new research directions in environmental biotechnology, ***only a few are working in this area today.***
- Fortunately, the number of chemical engineers showing interest is growing every year and with the recent research emphasis on biotechnology, it is easy to envision that many others will join this exciting research area in the near future.

Engineering Biosorbents for Heavy Metal Removal

- Immobilization of heavy metals into biomass or precipitation through reduction to lesser bioactive metal species, such as metal sulfide are the major mechanisms employed by nature (microorganism, animals and plants) to counteract heavy metal toxicity.
- These natural mechanisms can be easily exploited to ***optimize biosorbents*** that are more efficient for heavy metal removal in large scale.
- *Example, a sulfide-dependent metal removal strategy was developed by engineering **the sulfate reduction pathway** into a robust bacterium E. coli 2 and used in large scale.*
- The resulting strains *produced significantly more sulfide and removed more than 98% of the available cadmium under anaerobiosis*.

Biofuels Production

- *Biohydrogen, biodiesel and biogas production* through *anaerobic fermentation* is a sustainable alternative for managing the recent energy crisis and creating a sustainable green environment.
- Fermentative hydrogen production processes are technically feasible and economically cost competitive and have large-scale commercialization implications.
  * Microbes present in the sediments of mangroves have the capability to yield biohydrogen.
  * Mangrove sediments are inherently rich in organic content and offer advantages such as: flexible substrate utilization and the simplicity of handling, no major storage problems, minimal preculturing requirements and sediments being available at low cost.
- *The development of (bio)energy using marine and freshwater microalgae for 3rd generation:* biomass feedstock has also been explored recently because microalgae can grow fast with high specific growth rates and have excellent CO_2 absorption capacity and better regulation of lipid and sugar content under various culture conditions.

  SRT = 1/(specific growth rate) = $1/\mu$

Objectives of Environmental Biotechnology According to Agenda 21 in UN:

- ❖ Aim is to prevent, arrest and reverse environmental degradation through the appropriate use of biotechnology in combination with other technologies, while supporting safety procedures as a primary component of the programme.

Specific Objectives

1. To adopt production processes that make optimal use of natural resources, by recycling biomass, recovering energy and minimizing waste generation.
2. To promote the use of biotechnological techniques with emphasis on bioremediation of land and water, waste treatment, soil conservation, reforestation, afforestation and land rehabilitation.
3. To apply biotechnological processes and their products to protect environmental integrity with a view to long-term ecological security.

- ❖ ***Generally, researches should be done on:***
- ➢ Different organisms need different types of nutrients, for example, certain bacteria thrive on the chemical components of waste products, whereas some other microorganisms feed on materials toxic to others. Thus, detail study is required.
- ➢ Despite escalating efforts to prevent waste accumulation and to promote recycling, the amount of environmental damage caused by over-consumption, the quantities of waste generated and the degree of unsustainable land use appear likely to continue growing.
- ➢ The remedy can be achieved, to some extent, by the application of environmental biotechnology techniques includes bioremediation, prevention, detection and monitoring, genetic engineering for sustainable development and better quality of living.

Environmental Biotechnology Challenges and Perspectives:

- New environmental challenges continue to evolve and new technologies for environmental protection and control are currently under development.
- Also, new approaches continue to gain more and more ground in practice, harnessing the potential of microorganisms, plants and animals together with photons as eco-efficient and robust cleanup agents in a variety of practical situations such as :
  * Enzyme engineering for improved bio-degradation
  * evolutionary and genomic approaches to bio-degradation
  * designing strains for enhanced bio-degradation
  * process engineering for improved bio-degradation
  * re-use of treated wastewater
  * bio-membrane reactor technology
  * design wastewater treatment based on decentralized sanitation and reuse
  * implementation of anaerobic digestion to treat bio-waste
  * bio-development of bio-waste as an alternative and renewable energy resource
  * emerging and growing-up technological applications of soil remediation and cleanup of contaminated sites.

Wastewater Treatment

❖ ***Waste*** is divided into three: ***liquid, solid and gasses*.**

▪ ***Wastewater*** is commonly used to describe liquid wastes that are collected and transported to a treatment facility through a system of sewers.

❑ ***Wastewater*** can be classified into ***domestic (sanitary)*** *and* ***industrial*.**

a) ***Domestic wastewater*** comes exclusively from residences, institutions and business buildings such as schools, universities, hospitals, hotels, etc. ***It has 99.9% water & 0.1% solids.***

* The solids in domestic wastewater are both dissolved and suspended solids.

Suspended solids can be settled out or filtered but dissolved solids will have to be converted to suspended solids during the treatment process.

b) ***Industrial wastewater*** comes from manufacturing plants.

❖ ***Assurance of water quality*** has become an *integral part of environmental quality management* in the world today including Ethiopia.

▪ ***Thus, wastewater treatment is one of the strategies for water quality management.***

➢ ***Some fundamental reasons for treating wastewater:***

- Prevent or reduced pollution and protecting the environment; and,
- Protecting public health and ecosystem by safeguarding soil, air and water supplies and preventing the spread of air and water-borne diseases.
- Contribute on solving shortage of water by recycling the treated water.

❖***The industrial processes generate different types of wastes:*** solid, soft, liquid or gaseous that can be ***corrosive, reactive, explosive and toxic***.

❖ ***These residues pose a risk to human health and environment/ecosystem, such as*** *infectious diseases transmitted in excreta, Biodegradable organic wastes for gaseous emission*, heavy metals, etc.

❖ **Wastes can be broadly grouped into two**:

(a) The Biodegradables (Biowastes): include those solid wastes generated, which could be decomposed by microorganisms and does not constitute major sources of pollution for a long period of time.

- These are *paper products* (such as printing papers, waste books, newspapers, carton, toilet paper, card boards), *wastes of plant origin* (fruits, stems, roots, vegetables, leaves, food remains and garden solid wastes, etc.) and *wastes of animal origin* (faecal matter, carcass, droppings, and poultry waste products).
- Give off offensive odour and constitute nuisance to the aesthetic environment more than the nonbiodegradable solid wastes.
- Constitute a good habitat for the thriving of pathogenic microorganisms and pests which could easily pollute fresh food product and sources of fresh water in the urban cities.

(b) Nonbiodegradable (Rubbish/Garbage): groups of solid wastes are not degradable or hardly degraded by microorganisms, and means of treatment includes incineration, recycling, etc

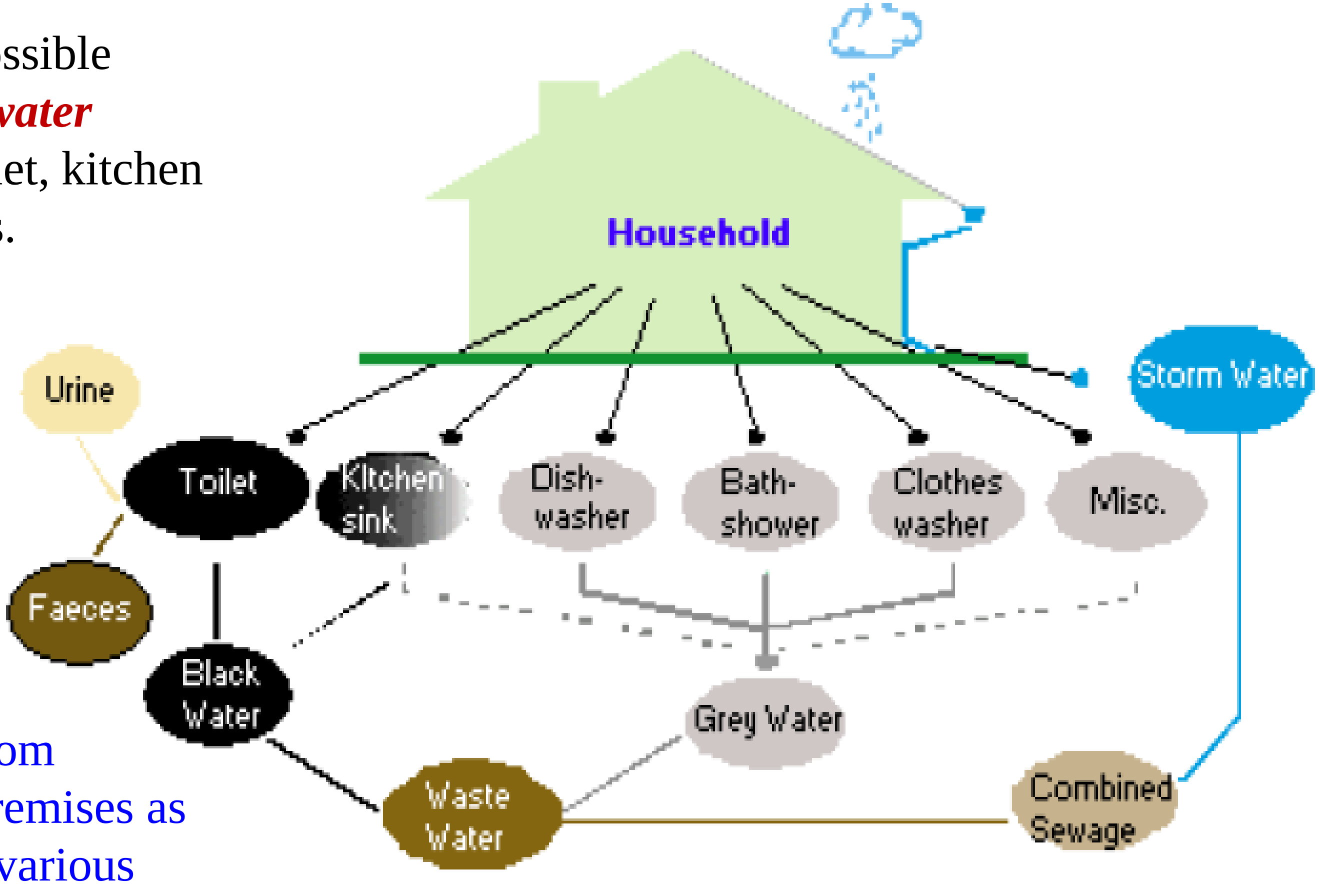
- These are even worse nuisance to the environment since their disposal has become a herculean and near impossible task in most countries.

✓ ***Some examples of Nonbiodegradable***

- solid wastes of metallurgical and smelting industries (abandoned vehicles, and vehicle part and scrap metals, iron, zinc, aluminium sheets and others);
- solids wastes of construction industries (waste building materials);
- solid waste of plastic industries (plastic buckets, cable insulators, tyres and glass products.

❖The major problem in Ethiopia is yet to develop efficient ways of waste disposal which are eco-friendly like recycled back into new product without constitute nuisance to the environment or affecting the health of the biotic components of the ecosystem.

➢ Figure shows a range of possible ***sources of household wastewater*** showing wastewater from toilet, kitchen Bathroom, laundry and others.

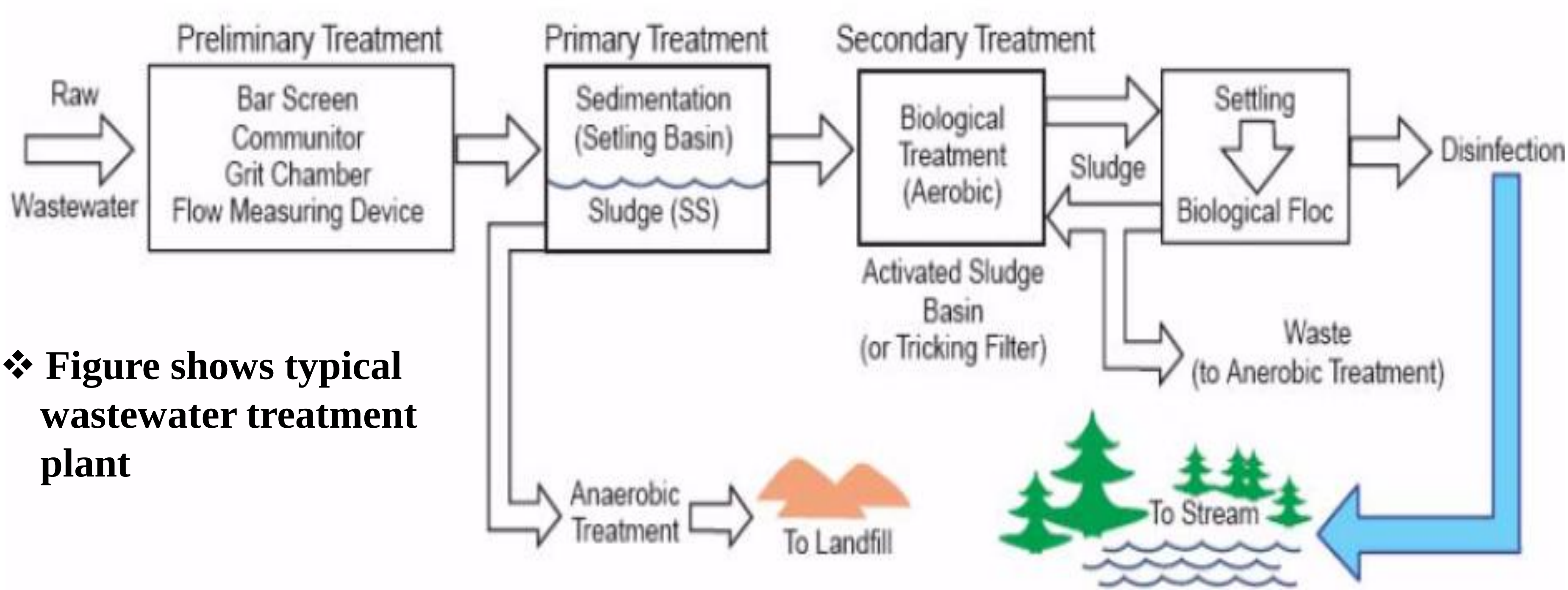


▪***Organic waste originates*** from domestic and commercial premises as ***sewage***, from urban runoff, various industrial processes and agricultural activities.

- ***Sewage*** is a complex mixture of natural inorganic and organic materials.
- The main source of pollution in sewage is ***human excreta*** with smaller contributions from food preparation, personal washing, laundry and surface drainage.
- The ***chemical*** and ***physical*** nature of wastewaters can be further ***complicated*** by the inclusion of ***industrial wastes*** which are composed of **strong spent liquors** from main industrial processes and comparatively weak wastewaters from rinsing, washing and condensing.

Basic Wastewater Treatment Processes

- A wastewater treatment is a combination of separate treatment processes or units designed to produce an effluent of specified quality from a wastewater of known composition and flow rate and required to process the separate solids and liquid to a suitable condition for disposal.
- The amount of treatment required depends largely on the water quality objectives for the receiving water and the dilution available.



❖ **Figure shows typical wastewater treatment plant**

➢ Industrial wastewater-variety of pollutants with varying concentrations and properties

- No single technology available to treat all industrial wastewaters
- Technology selection-based on type of pollutants, concentration of pollutants and treated water quality requirement
- ***Treatments: A combination of Physical, physico-chemical and biological processes***

A) ***Physical Processes***

- Sedimentation
- Surface filtration, Sieves, cloth filters, membrane filters, Deep filtration
- Sand and other media filters
- Evaporation: natural, vacuum and mechanical evaporators
- Gas Transfer

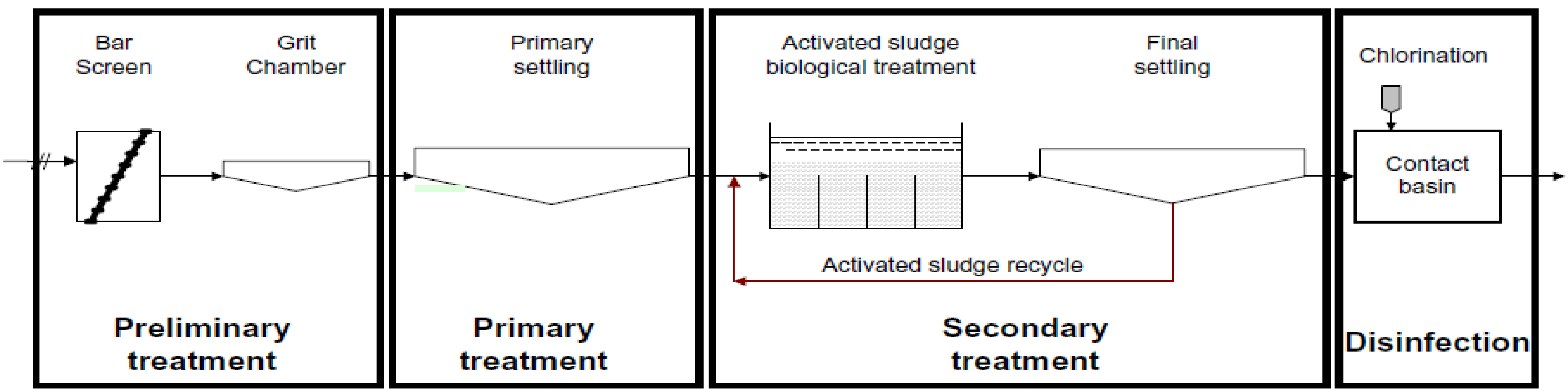
B) ***Physico-Chemical Processes***

- Coagulation and flocculation, electro coagulation
- Adsorption-New and tailor made adsorbents
- Ion Exchange -
- Precipitation
- Membranes –RO, CDI, electro dialysis
- Oxidation Reduction
  –Advanced Oxidation

C) ***Biological Processes***

- Aerobic
- Anaerobic
- Biological processes can be modified by using enriched microbes for selective complex organic wastes.

❖ **Figure shows Typical wastewater treatment plant**



❖ ***Unit treatment processes can be classified into five stages:***

(1) **Preliminary treatment**: the removal and disintegration of gross solids, and oil and grease are also removed at this stage if present in large amounts.

(2) **Primary (sedimentation) treatment:** the first major stage of treatment following preliminary treatment, which usually involves the removal of settleable solids which are separated as sludge.

(3) **Secondary (biological) treatment:** the dissolved and colloidal organics are oxidized in the presence of microorganisms.

❖ The three basic types of organisms important to the operation of an activated sludge system are **microbes** such as bacteria, fungus, etc, **plants** like algae and **aquatic animals** such as protozoa, crustacians and rotifiers, where by ***microbes are more preferred.***

(4) **Tertiary treatment:** further treatment of a biologically treated effluent to remove BOD5, bacteria, suspended solids, specific toxic compounds or nutrients to enable the final effluent to comply with a standard,

(5) **Sludge treatment:** involves the stabilization, dewatering/thickening, and disposal of sludge.

➢ ***Such treatment processes are required expensive cost, energy, chemicals, large spaces for construction, as a result led to inefficient treatment.***

▪ Consequently, developing countries will not afford such technology, instead required simple but effective methods such as implementation of ***Environmental Biotechnology***.

❖ Before recommending wastewater treatment methods, first it is necessary to know the ***composition and characteristics of the wastewater***.

Wastewater Characterization and Classification

- The sources of wastewater can be from municipal/domestic or industry.
  - Due to source difference, the composition and rate of concentration of the WW is different.
  - Thus, *classification and characterization of the wastewater in physicochemical* composition and biological aspect is important before recommending treatment methods.

- ***There are several ways to approach for classification of wastewater***

a) Through a list that gives qualitative description of wastes by type, source and waste component.

b) Waste definition through **standardized tests**, for example, WHO tests where the content of certain substances determines if the waste is hazardous

c) Waste definition through **concentration limits** within the same residue.

Composition:

- As shown in the following table, the composition of the wastewater to be treated is necessary to have as much knowledge as possible to:
  - Assess the pollution strength
  - Determine whether pretreatment is required;
  - Determine whether an industrial waste should be treated alone or with sewage and, if so, in what proportions;
  - Determine whether an industrial waste would attack the sewer;
  - Permit a better selection of the most appropriate treatment process;
  - Allow an assessment of the toxicity or disease hazards;
  - Provide indication of the resultant degree of eutrophication or organic enrichment in the form of sewage fungus in the receiving water (i.e. impact assessment); and
  - An assessment of the recoverable or reusable fractions of the wastewater.

Wastewater Characterization

- Characteristics of wastewaters can usually be represented by the basic parameters, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD5), suspended solids (SS), ammonium nitrogen (NH_4^+-N), heavy metals, pH, color, turbidity, biological parameters and others, and depends on your objective.
- ***Most characteristics of industrial wastewaters strongly depend*** *on the* ***type of industrial wastewaters and industrial processes***.

- Compared with municipal wastewater, ***industrial wastewaters*** usually have a **high organic strength and extreme physicochemical nature** (e.g., pH, temperature, salinity), and ***contain synthetic and natural substances*** that may be toxic to or inhibit biological treatment processes.
- E.g. ***Municipal wastewater*** is characterized by low organic strength (250–800 mg COD/L), whereas ***industrial operations*** often generate strong (*>1000 mg COD/L*) to extremely strong wastewaters.
- Extremely strong wastewaters, with ***COD concentrations*** that may even exceed **200 g/L**, are generated in *olive mills, textile and beverage production industries*.
- Most industrial wastewaters have ***non-neutral pH*** , and some industrial wastewaters such as from petroleum refining, textile processing, leather processing, and food conservation have ***high salt concentrations***.
- In general, ***extreme pH and salinity*** may give rise to difficulties in the biological treatment of industrial waste streams.
- ***Inhibition of many microorganisms and deflocculation of sludge flocs*** were frequently encountered problems when wastewater treatment systems were operated under these conditions.

Table 1.17. Comparison of typical chemical composition of raw wastewaters from the USA and UK.

Parameter	USA ($mg\ l^{-1}$)	UK ($mg\ l^{-1}$)
pH	7.0	7.2
BOD	250	326
COD	500	650
TOC	250	173
Total solids	700	—
Suspended solids	220	127
Total nitrogen	40	66
Organic nitrogen	25	19
Ammonia nitrogen	25	47
Nitrite	0	0
Total phosphorus	12	15
Oraganic phosphorus	2	3
Inorganic phosphorus	10	12

❖ The impurities in the waste water can be divided into ***suspended matter, colloid and dissolved matter according to the size of the particles.***

- Other large particles greater than 100 µm are generally precipitated naturally or separated from the water.
- Colloids and fine particles and suspended solids on the need for cohesion method, flocculation method, coagulation method to convert small particles into large particles, precipitation separation in the water.

Specific Limits

- A significant element in wastewater disposal is the potential environmental impact associated with it.
- Environmental standards are developed to ensure that the impacts of treated wastewater discharges into ambient waters are acceptable.
- Standards play a fundamental role in the determination of the level of wastewater treatment required and in the selection of the discharge location and outfall structures.
- Regulations and procedures vary from one country to another and are continuously reviewed and updated to reflect growing concern for the protection of ambient waters.
- ***Effluents discharged to receiving water bodies should achieved the following minimum wastewater quality limits:***

Parameter	Effluent Limit
BOD_5	20mg/l
TSS	30mg/l
Nitrates (as Nitrogen)	30mg/l
Phosphate	10mg/l
COD	100mg/l
pH	6 – 9
Faecal coliform	1000MPN/100ml
Residual chlorine	1.5mg/l

MPN- Most Probable Number

Important factors in the selection of wastewater treatment system

Urban Wastewater

- The strength of the wastewater is determined by measuring the amount of suspended material and organic material in the water.

a) *The suspended*, filterable solids in the waste flow are known as Suspended Solids or SS.

- They can be trapped on a filter, dried, and weighed to determine the concentration.

b) *The organic strength* of the wastewater is determined indirectly.

- The microorganisms in the biological treatment processes decompose or stabilize the raw organic material in the waste flow through the use of oxygen as part of the respiration process.
- Instead of directly measuring the strength of the organics, the amount of oxygen that the microbes use as they eat it is determined.
- This is known as the ***Biochemical Oxygen Demand 5 (BOD5) after 5 days incubation.***

❖ Fresh Sewage has a musty odour, a grey colour, and contains both organic material and sufficient dissolved oxygen to support the growth of aerobic bacteria.

- Aerobic bacteria, as do humans, need a food supply and a source of free oxygen to survive.
- The food supply is furnished by the organic material in sewage, and the free oxygen is available as dissolved oxygen (DO).
- The DO is depleted as the aerobic bacteria attack the organic material contained in the sewage.
- Some of the DO can also be depleted through chemical action.
- The sewage will become ‘stale’ and then ‘septic’ as DO is depleted.
- Septic sewage contains no DO, and all bacterial action will be anaerobic.
- Industrial non-organic wastes can also deplete oxygen in the water, and this is measured by the ***chemical oxygen demand (COD) test***.

- COD is a measure of the oxidisability of waste, expressed as the equivalent amount in oxygen of a strong oxidizing agent consumed by the waste under fixed laboratory conditions.
- The COD test uses the oxidising agent potassium dichromate to oxidise organic matter in the sample.
- The COD test is extensively used due to taking less time (about 3 hours) than other tests such as the BOD5, which takes 5 days. *Some of the suspended solids are organic*, but most of the organics in the wastewater are dissolved.
- *About 40% of the BOD will be suspended particles and most of them will settle out without further treatment.*
- *But 60% of the BOD is dissolved and must be used as "Bug Food" to grow a culture of microorganisms that become "suspended solids" that are again removed by settling.*
- ***Suspended solids are classified as "Suspended" and "Settleable".***
- About 60-70% of the Suspended Solids in raw wastewater are settleable.
- Domestic wastewater will have a **BOD** of between **150-300 mg/L** and **SS** in the **100-400 mg/L range.**
- Nitrogen, in the form of ammonia, will also be present in domestic wastewater. Ammonia concentrations usually run from 10-40 mg/L.
- Hospitals and other medical facilities have the potential to discharge wastes that are *radioactive or represent a biohazard*.

Industrial Wastewater

- *Industrial wastewater characteristics vary with each type of discharge.*
- Some food process wastes like potato processing and some vegetable canning can have a high pH, whereas like green chili processing or fruit canning can have a low pH.

- Food processing, dairy operations and meat packing all have wastes that are very high in BOD, sometimes over 1000 mg/L.
- BOD is high in dissolved sugars, fats and proteins and ***low in suspended material***, but ***dramatically increase the loading on the secondary treatment processes***.
- Toxins, like heavy metals coming from metal plating, battery shops, and heavy equipment manufacturing, and solvents from industries like body shops, dry cleaners, and furniture manufacturing can kill organisms that are needed to treat the waste.
- Copper, chrome, lead, and cyanide are all chemicals that are commonly found in heavy industrial manufacturing discharges.

➢ These indicates, the presence of different pollutants in the wastewater makes it almost impossible to treat all the wastewater in the same manner.

❖ Thus, important to consider factors during selection of treatment technology (see table below)

Factor	Developed countries		Developing countries	
	Critical	Important	Critical	Important
Efficiency	x			x
Relaibility	x			x
Sludge disposal	x			x
Land requirements	x			x
Environmental impacts		x		x
Operational costs		x	x	
Construction costs		x	x	
Sustainability		x	x	
Simplicity		x	x	

❖ Table shows important factors in the selection of wastewater treatment system in developed and developing countries (von Sperling, 1995).

❖ The following table shows Important contaminants of concern in wastewater treatment.

Contaminants	Reason for importance
Suspended solids	Suspended solids can lead to the development of sludge deposits and anaerobic conditions when untreated wastewater is discharged in the aquatic environment
Biodegradable organics	Composed principally of proteins, carbohydrates and fats, biodegradable organics are measured most commonly in terms of BOD and COD. If discharged untreated to the environment, their biological stabilization can lead to the depletion of natural oxygen resources and to the development of septic conditions
Pathogens	Communicable diseases can be transmitted by the pathogenic organisms in wastewater
Nutrients	Both nitrogen and phosphorus, along with carbon, are essential nutrients for growth. When discharged to the water these nutrients can lead to the growth of undesirable aquatic life. When discharged in excessive amounts on land they can also lead to the pollution of groundwater
Refractory organics	These organics tend to resist conventional methods of wastewater treatment. Typical examples include surfactants, phenols, and agricultural pesticides
Heavy metals	Heavy metals are usually added to wastewater from commercial and industrial activities and may have to be removed if the wastewater is to be reused
Dissolved inorganic solids	Inorganic constituents such as calcium, sodium, and sulfate are added to the original domestic water supply as a result of water use and may have to be removed if the wastewater is to be reused

Source: Metcalf & Eddy, Wastewater engineering

Methods of Wastewater Sampling and Characterization

a) Wastewater Sampling Procedures

❖ If a waste is discharged to river, sample will be collected from the effluent at different distance : at the beginning, some distance from middle and end part of the effluent.

❖ If it is from collection ponds, collect the samples from the surface, middle and bottom part.

❖ Use sterilized glass bottle or plastic bottles for sample collection.

❖The plastic bottles should be rinsed overnight with 1M HCl and then with distilled water. Next, the bottles should also rinsed thrice with sample water before final collection. OR

▪ Prior to sampling, the 1-L polyethylene bottles will be cleaned by incubating them with 10 % (v/v) nitric acid solution for 48 hours in a hot water bath and then washed and rinsed with distilled and de-ionized water.

❖ Temperature and pH should be determined at the sampling site using pH meter and thermometer.

❖ BOD5, COD and others physical parameters are also possible to measure on the spot.

❖Collected samples should be placed in a cooler box with ice for transportation to the laboratory.

❖ Samples for heavy metal analyses are fixed by adding 2-3 drops of Nitric acid and stored at 4°C.

❖ Samples should be stored in a refrigerator at 4°C until analysis.

b) Wastewater Analysis Methods

❖ After samples are collected, the following physico-chemical parameters will be analyzed in the laboratory.

a) Electrical conductivity(EC), chemical oxygen demand (COD), biological oxidation demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), color intensity, chloride, sodium, sulphate, nitrate, phosphate and heavy metals ions and others.

b) Sample EC and TDS will be determined by conductivity meter, whereas color sample by spectrophotometer at appropriate wave length.

c) BOD will be determined by DO meter using evaporation methods whereas COD can be measured directly by COD instrument.

d) Chloride contents will be assessed by titrimetric whereas sulphate by turbidity methods

e) Heavy metals ions in the wastewater sample will be determined by atomic absorption spectrophotometer (AAS) after digested by acid whereas phenolic compounds will be measured by photometric methods.

a) Turbidity of the samples will be measured using Turbidimeter.

Types of Wastewater Treatment

1. Aerobic and Anaerobic Treatment Processes

Biochemical Environment

- ***Aerobic Conditions:*** Oxygen is used as electron acceptor
- ***Anoxic Conditions:*** Nitrate is the electron acceptor and reduced to N2
- ***Anaerobic Conditions:*** absence of oxygen and nitrate, instead used sulphates and reduced to sulphides (H2S), and CO2 is converted into methane and biomass production.

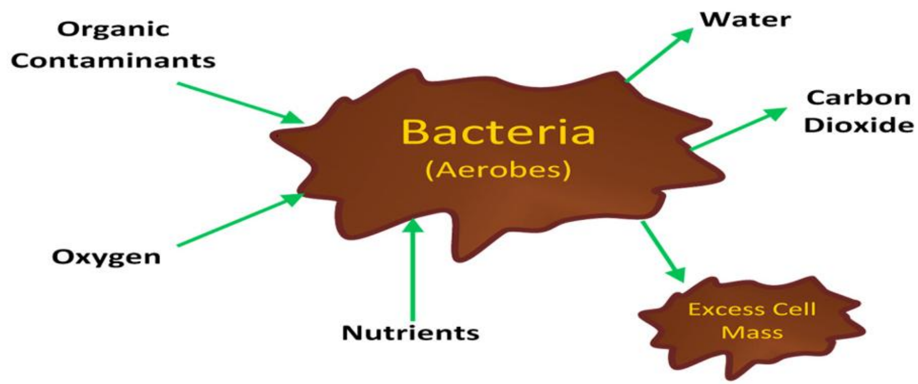
$$\text{Organic matter} + H_2O \longrightarrow CH_4 + CO_2 + NH_3 + H_2S + \text{new cell}$$

Aerobic Treatment Process

- Stable and efficient removal of the biological solids produced in biological reactors is critical to the operation of biological wastewater treatment systems for the production of high quality effluent.
- ***Activated sludge*** is the most common biological wastewater treatment technology used in industrialized countries, and in this process ***biological solids are removed by sedimentation***.
- Poor settling of biological solids remains one of the most common operational problems in activated sludge wastewater treatment systems around the world.
- This can lead to increased solids treatment costs, increased effluent solids concentrations, decreased disinfection efficiencies, and increased risks to downstream ecosystems and public health.
- ***Well-mixed reactor, suspended growth and a gas separator before settling tank*** is used to ***make settling easier as well as simple and inexpensive.***
- ***However, system is not stable for shock loading and toxic compounds and confront with settling is problem.***

Aerobic Treatment Systems

- Activated sludge Process (CSTR with/without cell recycle)
- Contact Stabilization
- Oxidation Ditch
- Sequencing batch reactor (SBR)
- Extended Aeration
- Membrane bioreactor (MBR)

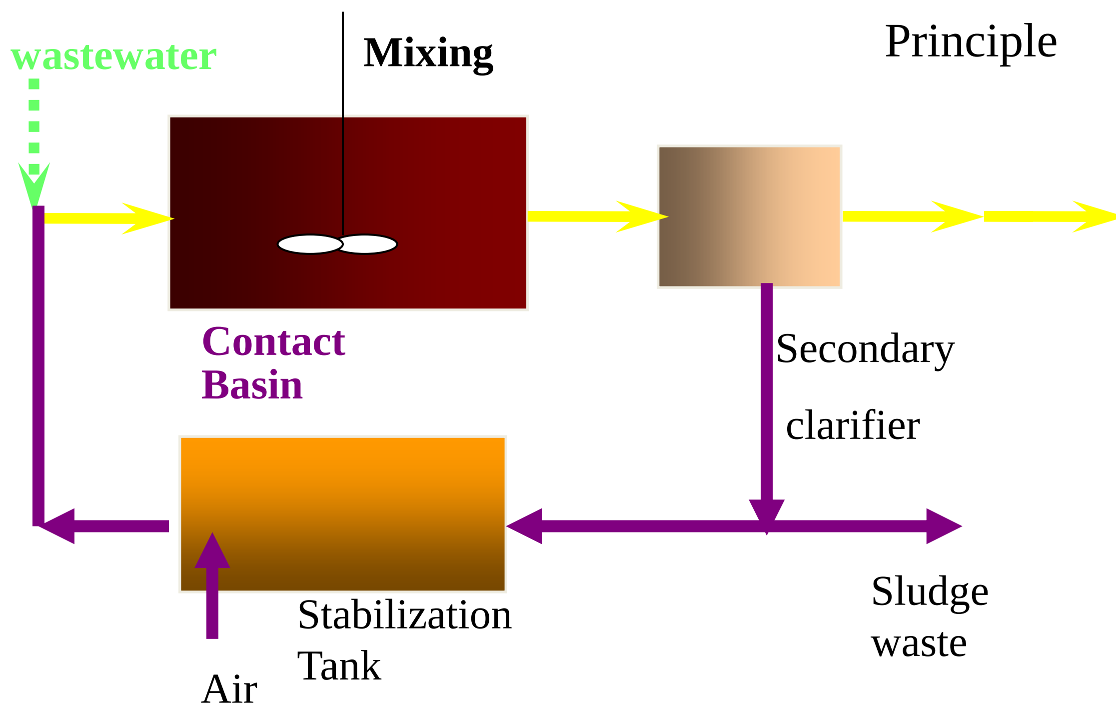


Sequential Batch Reactor

- No settling tank, no sludge pumping
- Aerobic/anoxic/anaerobic cycles for nutrient removal
- Process flexibility for bulking
- Tolerant to shock loading
- No washout

Contact stabilization

The figure shows Aerobic Treatment Principle



❖ ***The following table shows Comparison of Aerobic Biological Treatment Options***

Parameter	Conventional ASP	CASS™	IFAS	MBR
Treated Effluent Quality	Meets specified discharge standards with additional filtra-tion step	Meets/ exceeds specified discharge standards without additional filtra-tion step	Meets/ exceeds specified discharge standards with additional filtration step	Exceeds specified discharge stan-dards without additional filtration step. Very good for recycle provided TDS level permits
Ability to adjust to variable hydraulic and pollutant loading	Average	Very good	Very good	Very good
Pretreatment Requirement	Suspended impurities e.g. oil & grease and TSS removal	Suspended impurities e.g. oil & grease and TSS removal	Suspended impurities e.g. oil & grease and TSS removal	Fine screening for suspended impurities like hair and almost complete oil & grease removal
Ability to cope with ingress of oil	Average	Good	Average	Poor & detrimental to membrane
Secondary Clari-fier Requirement	Needed	Aeration Basin acts as clarifier	Needed	Clarifier is replaced by Membrane filtration
Complexity to operate & control	Simple, but not operator friendly	Operator friendly	Operator friendly	Requires skilled operators
Reliability & Proven-ness of Technology	Average	Very good	Very good	Limited references in industrial applications
Capital Cost	Low	Low	High	Very High
Operating Cost	Low	Low	High	Very High
Space Requirement	High	Low	Average	Low

Anaerobic Treatment Processes

❖ These two terms are directly related to the type of bacteria or microorganisms that are involved in the degradation of organic impurities in a given wastewater and the operating conditions of the bioreactor.

➢ In contrast to aerobic degradation, which is mainly a single species phenomenon, anaerobic degradation proceeds as a chain process, in which several sequent organisms are involved.

- Overall anaerobic conversion of complex substrates therefore requires the synergistic action of the microorganisms involved.

❖ It has been established that four physiological groups of bacteria are involved in the anaerobic conversion of organic materials to methane.

- The first and second groups of hydrolyzing and fermenting bacteria, converts complex organic materials to fatty acids, alcohols, carbon dioxide, ammonia and hydrogen.
- The third group of hydrogen producing acetogenic bacteria converts the products of the first group into hydrogen, carbon dioxide and acetic acid.
- The fourth group consists of methane forming bacteria, converting hydrogen and carbon dioxide or acetate to methane.

❖ The final products of organic assimilation in anaerobic treatment are methane and carbon dioxide gas and biomass.

➢ *The following figure shows Anaerobic Treatment Principle.*

❖ Anaerobic systems such as the Upflow Anaerobic Sludge Blanket (UASB), the Anaerobic Sequencing Batch Reactor (AnSBR) and the Anaerobic filter (AN) can successfully treat high-strength industrial wastewater as well as low-strength synthetic wastewater.

❖ Application of anaerobic systems for municipal sewage treatment is so far very limited. The predominant reason given for is, that municipal sewage are to weak (to low BOD or COD) to maintain high biomass (in the form of granules – suspended solids or fixed film) content in reactor.

- There are however, some successful examples in pilot and full scale.
  * Orozo (1997) investigated a full scale anaerobic baffled reactor (AnBR) to treat municipal sewage of an average BOD of **314 mgO2/L** for a hydraulic retention time of **10.3 hours**, (organic loading rate 0.85 kg/m3·d) and achieved a **70% removal efficiency**, that the process was run at very low temperature between 13 and 15 °C.

❖ The majority of anaerobic digestion plants are operated under mesophilic conditions (approx. 35 °C), however, most wastewaters are released for treatment at temperatures below 18 °C.

- Therefore many wastewaters are heated prior to treatment, thus consuming up to 30% of energy produced. Whereas, Low temperature causes deleterious effect on anaerobic digestion because of relatively longer generation time of anaerobic bacterial populations and lower biochemical activity, resulting in the decrease of biogas yield and digester failure.
- The start-up and treatment of municipal wastewater in cold regions was investigated in two UASB reactors operated at temperatures of 32, 20, 15, 11, and 6 °C with several HRTs ranging from 48 to 3 h (Singh and Viraraghavan, 1999).
- Biomass aggregation (granulation) was achieved in approximately 281 d at 20 °C.
- Psychrophilic (< 20 °C) anaerobic digestion has recently been proven feasible for the treatment of a range of industrial wastewater

➢ ***The following table shows Major Differences in Aerobic and Anaerobic Treatment***

Parameter	Aerobic Treatment	AnAerobic Treatment
Process Principle	• Microbial reactions take place in the presence of molecular/ free oxygen • Reactions products are carbon dioxide, water and excess biomass	• Microbial reactions take place in the absence of molecular/ free oxygen • Reactions products are carbon dioxide, methane and excess biomass
Applications	Wastewater with low to medium organic impurities (COD < 1000 ppm) and for wastewater that are difficult to biodegrade e.g. municipal sewage, refinery wastewater etc.	Wastewater with medium to high organic impurities (COD > 1000 ppm) and easily biodegradable wastewater e.g. food and beverage wastewater rich in starch/sugar/ alcohol
Reaction Kinetic	Relatively fast	Relatively slow
Net Sludge Yield	Relatively high	Relatively low (generally one fifth to one tenth of aerobic treatment processes)
Post Treatment	Typically direct discharge or filtration/ disinfection	Invariably followed by aerobic treatment
Foot-Print	Relatively large	Relatively small and compact
Capital Investment	Relatively high	Relatively low with pay back
Example Technologies	Activated Sludge e.g. Extended Aeration, Oxidation Ditch, MBR, Fixed Film Pro-cesses e.g. Trickling Filter/Biotower, BAF, MBBR or Hybrid Processes e.g. IFAS	Continuously stirred tank reactor/di-gester, Upflow Anaerobic sludge Blanket (UASB), Ultra High Rate Fluidized Bed reactors e.g. EGSBTM, ICTM etc.

Anaerobic Reactor Configuratios

- ***Anaerobic reactors*** present a unique ecosystem in which ***diverse groups of bacteria catalyze*** the conversion of complex organic compounds to methane and carbon dioxide in a highly controlled and coordinated fashion.
- Anaerobic degradation of organic matter is a complicated microbial process consisting of several interdependent consecutive and parallel reactions.

Advantages

- Process stability
- Produced Methane can be used to produce energy
- Produced amount of excess sludge is about 10 % of aerobic treatment. Hence, reduction of waste disposal cost
- Low nutrient requirement (BOD/N/P is 100/5/1 for anaerobic; 700/5/1 for aerobic MOs)
- No air supplementation, so lower operational cost; No off-gas air pollution
- Biodegradation of aerobic non-biodegradable
- Seasonal treatment is appropriate

Possible disadvantage

- Long startup
- Alkalinity should be sufficient
- Under mesophilic conditions, optimum temperature is 35 ^{o}C
- Nitrification not possible
- Low kinetic rates at low temperature
- ***If COD < 1000 mg/L anaerobic treatment is not practical economically***
- *Effluent from anaerobic treatment is generally not acceptable for direct discharge and aerobic polishing step is needed.*

2. Suspended and Anchored Cell Processes for Organic Wastes Removal

i) Suspended Cell Process

- **Floc** is clusters of microorganisms and solid particles that form in the activated sludge process and settle in the final clarifier.
- **Hydraulic Retention Time** is the period of time that wastewater remains in a tank., and is important because treatment processes require sufficient time for the wastewater to be treated.

❖ **Different processes are available, namely:**

a)Mixed Liquor Suspended Solids (MLSS)

❖The amount of suspended solids in an aeration tank, expressed in milligrams per liter (mg/L).

- MLSS consists mostly of microorganisms and non-biodegradable suspended matter.
- Total weight of MLSS in an aeration tank can be calculated by multiplying the concentration of MLSS (mg/L) in the aeration tank by the tank volume in cubic meter, and then multiplying the product by **8.34**.

b)Mixed Liquor Volatile Suspended Solids (MLVSS)

❖ MLVSS is the amount of organic or volatile suspended solids and *as a measure of the microorganisms present* in an aeration tank, expressed in mg/L.

- Total weights of MLVSS in an aeration tank can be calculated by multiplying the concentration of MLVSS (mg/L) in the aeration tank by the tank volume (TV), and then multiplying the product by 8.34.

c)Return Activated Sludge (RAS)

❖ RAS is the settled activated sludge (biomass) that is collected in a final clarifier and returned to the secondary treatment process to mix with incoming wastewater.

- This returns a concentrated population of microorganisms back into the aeration basin.

d) Organic Loading

❖ Organic loading is the amount of biodegradable material that exerts an oxygen demand on the biological treatment process.

▪ The organic strength of the wastewater is usually measured as **BOD5 in mg/L.**

▪ An organic overload is an event which significantly increases the organic loading (BOD) to the aeration basin above normal influent organic loading conditions.

Suspended-Growth Treatment Processes

➢ The most important suspended-growth biological treatment systems used for the removal of organic matter are *Activated sludge process, Aerated lagoons, Sequencing batch reactor and Aerobic digestion*.

Activated Sludge Process:

➢ In this method, the sewage containing organic matter with the microorganisms is aerated (by a mechanical aerator) in an aeration tank.

▪ The reactor contents are referred to as mixed liquor. Under aerobic conditions, the microorganisms metabolize the soluble and suspended organic matter.

▪ The generalized metabolic reaction is shown as follows.

$$\underset{\text{(organic matter)}}{COHNS} + O_2 + \text{Nutrients} \xrightarrow{\text{Bacteria}} \underset{\text{(new cells + other products)}}{CO_2 + NH_3 + H_2O}$$

❖ A part of the organic matter is utilized for the synthesis of new bacterial cells while the remaining gets oxidized to CO_2 , NH3 and H_2O.

❖ The newly formed microorganisms are agglomerated to form flocs, technically referred to as sludge.

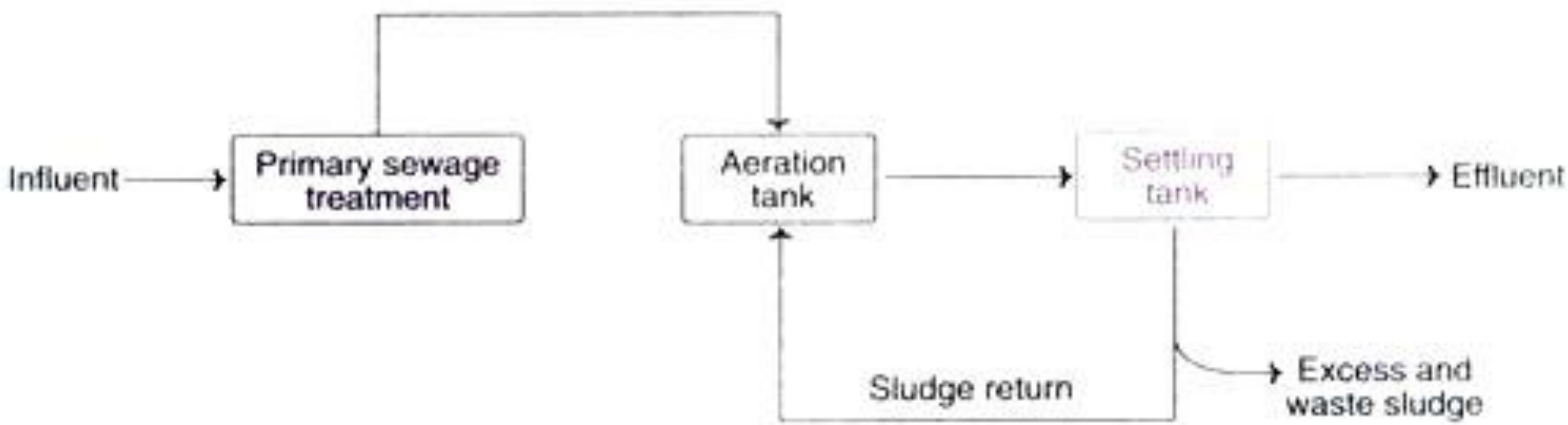
❖ The separated sludge which is not in contact with organic matter becomes activated.

- It is separated from the settling tank, and returned to the aeration tank, and recycled.
- The activated sludge recycled in aeration tank serves as a seed or inoculum, whereas the excess and waste sludge can be removed.

Factors affecting performance:

✓ There are several factors that influence the efficiency of activated sludge process, the most important being:

* the type of the reactor,
* aeration,
* food to microorganism (F/M) ratio,
* nutrients,
* sludge recirculation rate,
* pH and temperature.

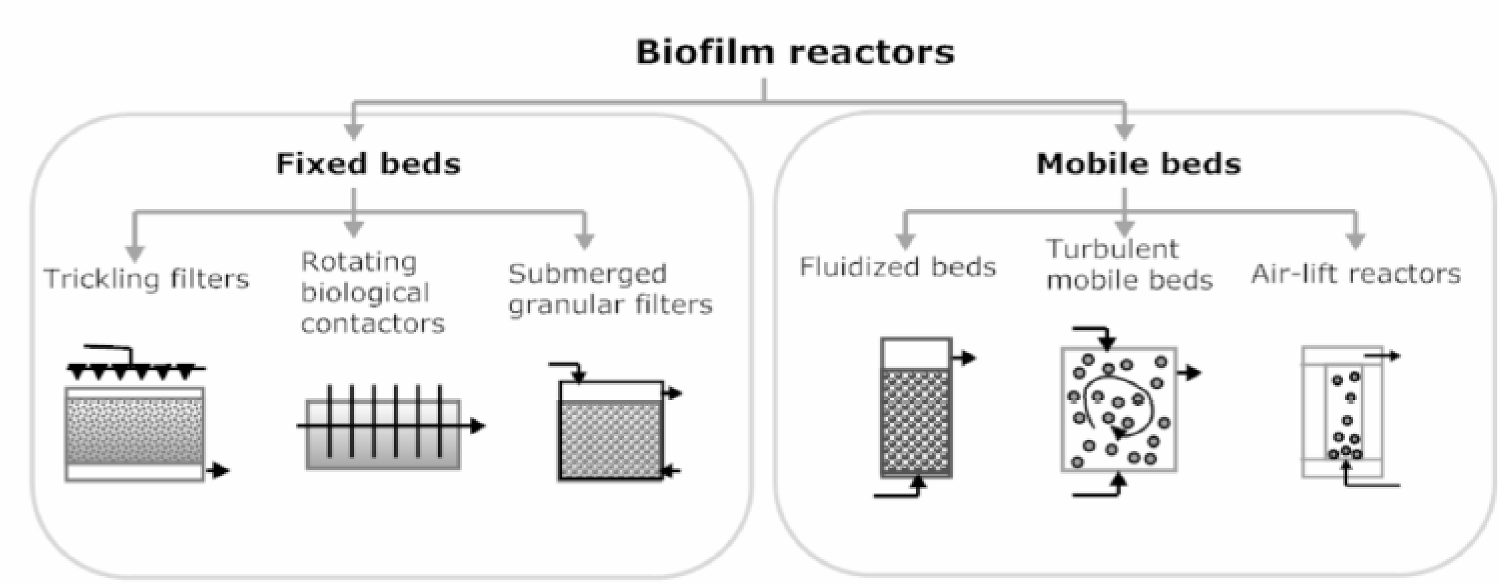


ii) Anchored Microbial Cell

- Effective use of organisms for enhanced nutrient removal in wastewater treatment applications requires the strains to be retained, to proliferate and to maintain biological activity within the process. This can be achieved by immobilization of the organisms using an appropriate system.
- For example: the increase of cells and chlorophyll "a" content showed that algae after immobilization are still able to undergo cell division and carry out photosynthesis.

Anchored in Biofilm Reactor

- Biofilm reactor configurations applied in wastewater treatment include *trickling filters, high rate plastic media filters, rotating biological contactors, fluidized bed biofilm reactors, airlift reactors, granular filters and membrane immobilized cell reactors*, as can be seen in the figure below. They used fixed and mobile beds for immobilization.



- Besides primary, secondary and tertiary wastewater treatment, biofilm systems have also been successfully used to treat industrial wastewaters.

- A general division between **fixed** and **moving** bed processes based on the state of the support material is usually done.
- ***Fixed bed systems*** include all systems where the biofilm is formed on static media such as rocks, plastic profiles, sponges, granular carriers or membranes.
  - * The liquid flow through the static media supplies the microorganisms with nutrients and oxygen.
- ***Moving bed systems*** comprise all biofilm processes with ***continuously moving media***, maintained by ***high air or water velocity or mechanical stirring***.
- ***Biofilm carrier material (media)*** is selected based on *size, porosity, density and resistance to erosion*.
- By using a material with a large specific surface area and high biological activity can be maintained using a relatively small reactor volume.
- The ***biofilm thickness in the reactors*** is usually controlled ***by applying shear force***, which is achieved by altering the stirring intensity, flow velocity or by backwashing.
- ***Biofilms used in wastewater treatment take advantage of a number of removal mechanisms such as biological degradation, biosorption, bioaccumulation and biomineralisation.***

Microalgae for wastewater treatment

- Secondary effluents from WWT plants contain nutrients (NH_4^+, NO_3^+ and PO_4^{3-}) which have been identified as the main causes of eutrophication in natural waters.
- Several types of unit processes exist for the removal of nutrients from wastewater but these are costly, technologically complicated and produce high sludge content.
- ***Microalgae*** have been proposed as an alternative biological treatment to remove nutrients using either ***suspended cells or attached*** (Mallick, 2002).
- *One of the limitations for the development of wastewater treatment systems based on microalgae is the harvest of the biomass at the end of the treatment process in **suspended way**.*
- ***The immobilization of cells*** can represent an alternative for solving the problem as well as providing advantages such as an increase in the cell retention time within bioreactors and higher metabolic activity.
- ***Immobilized cells** could be used as animal feed or as source of fertilizer and high-value chemicals if the biomass is harvest, along with nutrient removal from wastewater.*
- *Calcium alginate* is commonly used for immobilizing microalgae and maintains the high viability of cells for extended periods of time. *However, the matrix is vulnerable to the presence of chelating agents present in wastewater, such as phosphate and citrate, which affect the strength of the gel matrix and, ultimately, dissolves it.*
- Nevertheless, this problem can be resolved with the ***re-calcification of alginate beads.***
- Studies on nutrient removal from urban wastewater by immobilized microalgae are limited and include studies on *Chlorella* immobilized in alginate, *Scenedesmus obliquus* immobilized in k-carrageenan and *Scenedesmus intermedius* immobilized in calcium alginate.
- These studies have evaluated only the quality of the final effluent, and few have determined the nitrogen incorporation efficiency as protein, as well as the lipid content.

Routine algal culture and acclimatization

- ✓ The fresh water unicellular microalga like *Chlorella vulgaris* is used for tertiary wastewater treatment mainly for the removal of nitrogen and phosphorus compounds and heavy metals.
- ✓ *Chlorella vulgaris* will be isolated from agricultural soil and maintained as strains in the culture collection.
- ✓ For initial experiments, artificial wastewater (WW) with the following composition will be prepared (mg L^{-1}): NaCl, 7 mg; CaCl2, 4mg; MgSO47H2O, 2mg; KH2PO4, 15mg and NH4Cl, 115mg.
- ✓ These concentrations will be used simulating the mean values of the secondary effluent from the wastewater treatment plant: $N–NH_4^+$: 32.5 mg L^{-1}; $N–NO^{3-}$: 2.0 mg L^{-1} and $P–PO4^3$: 2.5 mg L^{-1}.
- ✓ Trace metals and vitamins will be added following guidelines for "f/2" medium preparation (Guillard and Ryther, 1962).
- ✓ During acclimatization (1 month), the microalgae will be transferred to fresh artificial WW every seven days.
- ✓ The artificial WW is only used for the acclimatization of cells and for direct comparison with real urban wastewater.
- ✓ Due to its nature, there is large variation in the concentration of nutrients so percentages of removal are used to determine removal efficiency.

Preparation of immobilized algal beads

- Once the microalgae are acclimatized, algal cells will be harvested by centrifugation at 3500 rpm for 10 min.
- The cells are resuspended in 50 mL of distilled water to form a concentrated algal suspension with a cell density of 10×10^{7} cells mL^{-1}.
- The algal suspension is then mixed with a 4% sodium alginate solution in 1:1 volume ratio to obtain a mixture of 2% algae–alginate suspension.
- The mixture will be transferred to a 50 mL burette and drops are formed when "titrated" into a calcium chloride solution (2%).
- This method produced approximately 6500 uniform algal beads of approximately 2.5 mm diameter with an initial cells number of 3.5×10^{5} cells $bead^{-1}$ for every 100 mL of the algae–alginate mixture.
- The beads will be kept for hardening in the CaCl2 solution for 4 h at 25 ± 2°C, then rinsed with sterile saline solution (0.85% NaCl) and subsequently with distilled water.
- A concentration of 2.6 beads per ml of wastewater (equivalent to 1:25 bead: wastewater v/v) will be placed in bioreactors made of transparent polyethylene terephthalate (PETE) containing 2.5 L of artificial wastewater or urban wastewater.
- The approximate volume of each bead will be 0.01538 mL.
- Check for removal efficiency of organic wastes.

3. Biological Removal of Organic Wastes

Mechanisms of Biological Removal

1. *Metabolic Basis*--The pollutants are served as food to microbes.
2. *Microbial Ecology and Its Control*--Create the conditions to select for the right MOs.
3. *Biomass Retention*--Develop systems to take advantage of natural aggregation as flocs and biofilms.

1. Metabolic Basis

- The principle: a pollutant to us is a substrate to some microorganism.
- Substrate means a material involved in generating energy to grow and sustain the MOs population. It is like "food" or "fuel."
- Substrate, fuel, or food involves sending electrons from an electron donor to an electron acceptor.
- Virtually every pollutant is an electron donor or an electron-acceptor for some group of MOs.
- A substrate can be a true fuel, or a source of energy that we can capture.

Examples of Treatment

Pollutant/Role	What We Add
Biodegradable organic matter (BOD)/donor	Acceptor: e.g., O2
Ammonium/donor	Acceptor: O2
Nitrate/acceptor	Donor: organic compound or H2
TCE/acceptor	Donor: H2 or organic H2 source

Examples of Energy-Capture

Pollutant/Role	Energy Outlet
• Biodegradable organic matter (BOD)/donor	• Methane (CH_4)
• Biodegradable organic matter (BOD)/donor	• Biohydrogen (H_2)
• Biodegradable organic matter (BOD)/donor	• Electricity (*i*)

2. Microbial Ecology and Its Control

- Deal with large, open systems. Microorganisms continually enter most processes.
- Have only partial control of the type and concentration of pollutants (fuels) that are input.
- Pure culture is not a relevant concept in practice.
- ▪ We deal with mixed cultures that often change.
- Therefore, the "game" is microbial ecology and steering it towards the types of microorganisms that do the job we want to be done.

Microbial Ecology as Science

❖ *As a scientific discipline, microbial ecology tries to answer these questions:*

- Who is there? (Community structure)
- What could they do? (Community potential)
- What are they doing? (Community function)
- What are their interactions with each other and their environment? (Community interactions)

Tools to Control the Microbial Ecology

- Adding the proper "other" substrate in the right amount
- Modest adjustments to pH, temperature, and nutrients, etc
- Efficient retention of biomass in general and the most critical microbial types in particular

3. Biomass Retention

❖ Biomass is retained because it **aggregates into** ***suspended flocs or attached biofilms*****.**

- ***Aggregation*** is a natural process that involves the ***production of extracellular polymeric substances*** (**EPS**), which are ***complex polymers*** involve protein, phospholipids, carbohydrate/polysaccharides and nucleic acid.

❖ Such aggregation of microbes formed due to the formation of **Biofilms,** which are currently identified as an assemblage of surface-associated microbial cells that are enclosed in hydrated **EPS.**

Solids Retention Time (SRT)

➢ **SRT** is the "master variable" for most environmental biotechnologies.

- It has **units of time** (e.g., days) and is the average time that active biomass is in the system.
- SRT = (total active biomass)**/(**net rate of active biomass production)

❖ Microorganisms remove pollutants from soil or water by absorbing and using the chemicals for their growth and development.

❖ ***Different methods by which microbes restore the environment are***

a) oxidizing and/or reducing
b) binding
c) immobilizing
d) volatizing and
e) transformation of pollutants.

❖ ***This is called Bioremediation and Biodegradation.***

Mechanisms of Biological Removal of Organic Nutrients

❖ The conventional physicochemical methods, such as *incineration, physical handling, isotopic destruction of pathogens and chemical treatment* of organic wastes suffer from one or the other drawbacks.

❖ **Chemicals** are known to ***reduce pathogens*** with ***very little effect*** on ***the total organic content of the waste***, but, microbial degradation is an efficient method for the disposal of organic wastes as they can reduce the total organic content of the waste.

➢ ***Biological treatment processes are systems that use microorganisms to degrade organic contaminants from wastewater,*** and are the preferred way of treatment as they are cost effective in terms of energy consumption and chemical usage.

- Example: **Biological Nutrient Removal (BNR)** is one approach for nutrient removal.
- **BNR** processes involve modifications of biological treatment systems so that the MOs in these systems can more effectively convert nitrate nitrogen into inert nitrogen gas and trap phosphorus in solids that are removed from the effluent.
- Example: ***Anaerobic digestion process*** to remove organic pollutants in O2 free system (see scheme in the next slide).

❖ ***Biological nutrient removal using microbes possible through:***

*** Assimilation**

- C, N, P, S etc uptake for **synthesis** of new cells.

*** Dissimilation**

- C, N, S, oxidized/reduced to provide **energy**

➢ ***These processes can be done using the following reactions:***

- **Aerobic**
  - in the presence of molecular oxygen (O_2)
- **Anoxic**
  - very low concentration of molecular oxygen (O_2)
  - significant levels of electron acceptors (NO_3^-, SO_4^-)
- **Anaerobic**
  - no oxygen, lack of electron acceptors (only CO_2)

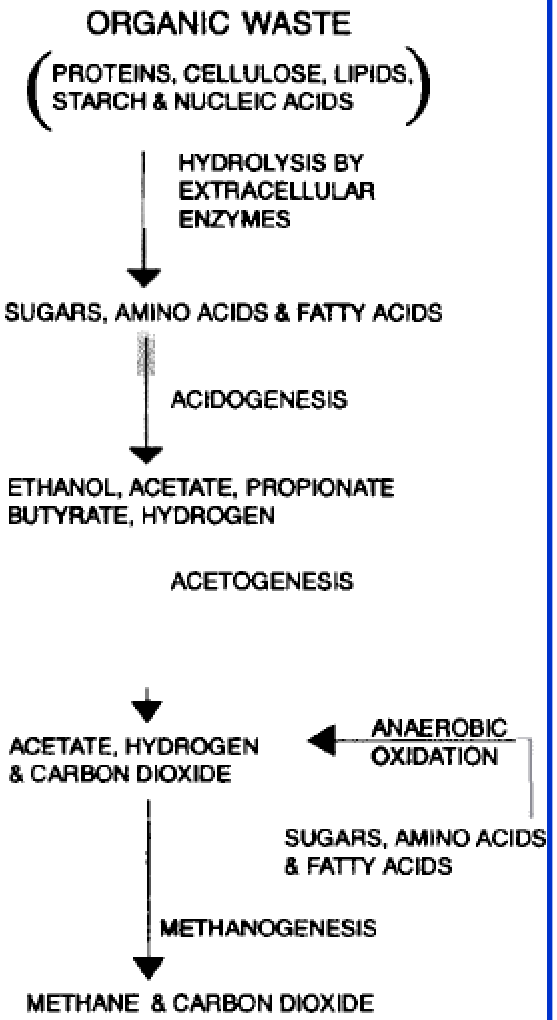
❖ The principle organic substances found in any organic wastes are proteins (40-60 %), carbohydrates (25-50 %), fats and oils (10 %).

❖ In most biological sewage treatment systems, the principle biochemical reactions that occur during the active waste stabilisation process are:

(a) Hydrolysis
(b) Oxidation/Reduction
(c) Cell synthesis/biomass generation
(d) Endogenous respiration

➢ *Microbial degradation of organic-wastes mainly comprising animal and human wastes, plant wastes, slaughter houses wastes, brewery wastes, agroprocessing wastes, etc are changed to biomass, intermediate metabolic products, biogas and compost as well as CO2, H2 and others.*

Scheme shows Steps involved in anaerobic digestion (methanogenesis) of organic waste.



Factors Influencing Biological Treatment

- Numerous factors are influences the choice and efficiency of biological waste treatment methods.

1) The type/suitability of the waste

* ***composition:*** *only organic wastes* of relatively *low toxicity can be treated.*

* ***physical form*** *such as size and moisture content:* biological processes *cannot be used for dry wastes such as dusts, without the addition of moisture to support microbial activity*.

2) Environmental conditions

* Biological treatment processes must be conducted within the *correct temperature range, appropriate moisture level, acidity or alkalinity, with the right level of aeration and nutrients* and *absence or relatively low inhibitors such as metals, chemicals etc.*

3) Type of MOs to be used

* *Selecting appropriate microbes based on the composition*, *growth environmental factors, the capability to ensure good contact between the organic constituents of the waste and the microbial population, etc are a key factor to considered.*

Process Conditions for Biological Treatment

1) Temperature limits – for the appropriate microbes to live, the temperature must be within the appropriate range. These are usually divide into psychrophilic (-5 to 10^{o}C), mesophilic (25-40^{o}C) and thermophilic (> 45^{o}C) ranges.

2) Minimum moisture and aeration are always necessary, and factors such as ***aeration*** influence the level of ***moisture needed.*** However, for wastewater treatment, which is the most common application for biological treatment, minimum moisture levels are not a concern.

3) pH limit: the capability of the different microbial species to break down organic wastes depends to a large extent on the ***pH of the medium 5 to 10***.

* Example, *Methanogens* operate between *pH 6 and 8*.

* When there is a risk of changes to the acidity, it is advisable to regularly measure and control the pH.

4) Oxygen limit:

a) Aerobic processes can only take place in presence of ***free oxygen*** from the air or aeration.

b) Anaerobic processes, ***oxygen is an inhibitor***. Example: ***Methanobacterium, Methanosarcina***

5) Presence of inhibitors:

* *Biological processes can be adversely affected by the presence of inhibitors* such as ***metals*** (Cu, Pb, chromium, etc) or ***chemicals*** such as pesticides which may be toxic to the microbe.

- Thus, the waste should not have ***inhibitors,*** instead should contain ***the nutrients*** useful for the growth of the microbes.

❖ It is almost always possible to find microbes suited to a given waste but these may be specialised microbes and not ubiquitous.

- For example, it is possible to select a bacterial flora able to live in the tanneries effluent, which are rich in chloride, sulphide and chromium salts, notorious toxics for most bacteria.
- Halophilic and halotolerant microorganisms are able to thrive and grow in saline and hypersaline environments.

❖ The number of active bacteria or fungi depends on the nutrients, and when the nutrient levels decrease, the microbes also decrease.

- As a result, 95% efficiency is generally considered the upper limit for a biological treatment process.

❖ Types of microbes involved in the ***activated sludge process*** include members of the genera:

Pseudomonas, Zoogloea, Achromobacter, Flavobacterium, Nocardia, Bdellovibrio, Mycobacterium, and

* the two most common nitrifying bacteria, *Nitrosomonas and Nitrobacter;* as well as

* various filamentous forms of bacteria and fungi, such as *Sphaerotilus, Beggiatoa, Thiothrix, Lecicothrix and Geotrichum*, may also be present.

4. Biological Nitrogen Removal

❖ ***What are the forms of nitrogen found in waste water***?

- In raw domestic wastewater, **nitrogen** exists primarily as ***organic nitrogen and ammonia nitrogen (Ammonia (NH_3) and ammonium (NH_4^+)).***
- In biological treatment processes, the organic nitrogen is quickly changed to ammonia nitrogen by natural processes. These compounds comprise a large portion of the total nitrogen found in the water sample.
- The sum of **organic nitrogen (40%)** and **ammonia nitrogen (60%)** is referred to as ***"Total Kjeldahl Nitrogen" (TKN).***
- Over a period of time, these forms in aerobic environment would be transferred to **nitrate-N.**
- ***The nitrate ion is the most oxidized form of nitrogen*** and is ***chemically unreactive in dilute*** aqueous solutions.
- ***Nitrate*** is a nutrient which serves as a fertilizer and the growth of aquatic plants can thrive in its presence.
- In certain water bodies, it is necessary to limit the amounts of fertilizing nutrients such as nitrates and phosphates which may be discharged in treated effluents to prevent excessive growths of algae and aquatic plants.
- Through a process known as ***eutrophication***, such excessive growths result in large masses of algae and nuisance plants that can cause serious harm or even destroy the aesthetic value of a body of water and mass kill of fishes.

➢ ***The need for removal of nitrate nitrogen from treated effluents is largely confined to locations where nutrient content is critical for the receiving stream.***

Effects of Nitrate Contamination

- **Nitrate** is found in most of the natural waters at moderate concentrations but **Nitrate** contamination in groundwater resources originates mainly from the excessive use of fertilizers and uncontrolled on-land discharges of raw and treated wastewater.
  - This can cause potential health hazards to ***infants and pregnant women***.
  - *This is due to the potential **reduction of nitrate to nitrite ion**, in the stomach of infants, which can **bind with the haemoglobin of the affected babies**, thus **diminishing the transfer of oxygen** to the body's cells resulting in a bluish skin colour often called **methaemoglobinemia** or the blue baby syndrome (Benefield et al., 1982).*
- The common conventional processes used to eliminate nitrate from water are ***ion exchange, reverse osmosis and electro-dialysis***.
  - The utility of these processes has been limited due to their expensive operation and subsequent disposal problem of the generated nitrate waste brine.
  - This leads to an increasingly important problem, limiting the direct use of the groundwater resources for human consumption in several parts of the world including Ethiopia.

❖ ***Over all effects of N and P in receiving waters?***

a) Increases aquatic growth (algae)
b) Increases DO depletion
c) Causes NH_4 toxicity
d) Causes pH changes

❖ ***Why is it necessary to treat the forms of nitrogen?***

- Improve receiving water bodies quality
- Increase chlorination efficiency
- Minimize pH changes in plant & aquatic animals
- Increase suitability for reuse
- Prevent NH_4 toxicity
- Protect groundwater from nitrate contamination

❖ ***Mechanisms of ammonia nitrogen from treated effluents affect receiving water bodies:***

1. Ammonia exerts an oxygen demand on the receiving stream. Thus, if an excessive amount of ammonia is present in an effluent, the oxygen content in the receiving stream can be depleted below that required by water quality standards.
2. Excessive amounts of ammonia can be toxic to biological life in the receiving stream.
3. Ammonia reacts with chlorine to form chloramines which can interfere with disinfection.

❖ Therefore, treatment of wastewater is necessary to reduce the impact of nitrogen waste on human health, particularly in infants, pregnant women and other biotic ecosystems.

❖ The extent and purpose to which ***nitrification and denitrification*** are applied are dependent on the level of nitrogen removal required and the specific treatment method(s) used.

❖ Removal of nitrogen from wastewater effluents is vital by ***biological means*** which is typically categorized into the two processes of ***nitrification and denitrification***.

Mechanisms of Biological Nitrogen Removal

1. Nitrification

- ***Nitrification*** is an **aerobic process** in which bacteria ***convert ammonia nitrogen to nitrate nitrogen*.**
- *Nitrification is applicable where effluent quality demands a reduction in ammonia to a specified level.*
- This process is achieved biologically by specific bacteria that oxidize ammonia to nitrite and hence oxidize the nitrite to nitrate.
- The conversion of ammonia to nitrate is primarily accomplished in a **two-step process** by nitrifying bacteria named *Nitrosomonas and Nitrobacter*.
- These two types of bacteria are both ***autotrophic*** and they each have a specific role in the two-step process.

a) Nitrosomonas converts ammonia to nitrite in the first step and

b) Nitrobacter subsequently converts the nitrite to nitrate in the second step.

- The stechiochemistry of Nitrification

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\textit{Nitrosomonas}} NO_2^- + 2H^+ + H_2O$$

$$NO_2^- + 0.5\ O_2 \xrightarrow{\textit{Nitrobacter}} NO_3^-$$

2. Denitrification

- Denitrification is a process in which several groups of ***bacteria convert nitrate to nitrogen gas*** which is once released to the atmosphere, applied following nitrification processes.

❖In this process, nitrate is converted to gaseous forms of N2 that released to the atmosphere, but small amount of nitrous oxide (N2O) or nitric oxide (NO) can also be produced.

$$\mathbf{NO_3^- + CH_2 + H^+ \xrightarrow{\text{denitrifying bacteria}} N_2\uparrow + CO_2 + H_2O}$$

- Some of groups of bacteria involved includes: Pseudomonas, Micrococcus, Bacillus, Archromobacter; all of which are ***heterotrophic*** and occur naturally in domestic wastewaters.
- These bacteria convert nitrates to nitrogen gas via a process known as ***nitrate dissimilation*** in which the chemically-bound oxygen in nitrate replaces molecular dissolved oxygen (as found in aerobic processes) in the respiratory processes of the bacteria.
- ***Denitrification is commonly categorized as an anaerobic process because of lack of dissolved molecular oxygen.***
- However, it is more accurate to characterize it as an ***anoxic process*** because of the bacteria's ability to use ***chemically-bound oxygen*** in nitrate to facilitate their respiratory processes.
- It occurs when the oxygen concentration in the wastewater becomes low enough that the bacteria begin to utilize ***nitrate as an electron acceptor under*** **anoxic conditions**.

❖ The specific forms of nitrogen which are important in wastewater treatment are: $\mathbf{N_2}$ (Nitrogen gas), $\mathbf{NO_2^-}$ (Nitrite), $\mathbf{NO_3^-}$ (Nitrate), $\mathbf{NH_3}$ (Ammonia), $\mathbf{NH_4^+}$ (Ammonium Ion), and Organic Nitrogen. The stechiochemistry of Denitrification:

A) Nitrification

1. $NH_4^+ + 1.5\,O_2 \xrightarrow{\textit{Nitrosomonas}} NO_2^- + 2H^+ + H_2O$

2. $NO_2^- + 0.5\,O_2 \xrightarrow{\textit{Nitrobacter}} NO_3^-$ (nitrified effluent)

B) Denitrification

$NO_3^- + CH_2 + H^+ \longrightarrow N_2\uparrow + CO_2 + H_2O$

denitrifying bacteria (many)

❖ ***Enzymatic reactions*** involved in denitrification in bacteria.

- All enzymes are located within or on the surface of the inner membrane.
- The enzymes involved are nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR) and nitrous oxide reductase (N2OR).

Factors affecting rate of Nitrogen removal

❖ Proper conditions must exist for N removal by both process to be enacted efficiently.

a) Carbonaceous Organic Matter
b) Dissolved Oxygen
c) Alkalinity
d) pH
e) temperature
f) Plug Flow Conditions
g) Sludge Age/Mean Cell Residence Time
h) Toxicity and others

- E.g.: The growth rate of these microbes reached a maximum at a **pH of 7.8.**
- Nitrification rate is decreases with decreasing temperature over a normal range of operating temperature.

5. Biological Phosphorus Removal From Wastewater

❖ The protection of water bodies such as lakes and dams from the abnormal growth of monocellular algae is of increasing concern. (E.g. Algal bloom)

▪ Removal of the nutrients, carbon, nitrogen and phosphorous ***singly and in combination*** from wastewater should be considered.

▪ Phosphorous removal is the preferred way of controlling ***algal blooms*** and the ***growth*** of other ***undesirable aquatic vegetation***.

➢ ***Why is it necessary to remove P from Wastewater? In summary:***

- Reduce phosphorus, which is a key limiting nutrient in the environment/ecosystem.
- Improve receiving water quality by:
  - Reducing aquatic plant growth and DO depletion
  - Preventing aquatic organism kill
- Reduce taste and odor problems in downstream drinking water supplies

❖ **On the contrary,**

▪ **Since *No living organism can reproduce without phosphorous, a central component of the nucleic acids, careful planning of removal of P should be designed*.**

▪ *All energy consumption and energy production by living organisms require phosphorous.*

▪ For example: The phosphorus content in bacterial cells is usually around **1-3 % of the dry** weight while the corresponding percentage for PAOs can reach **10%**.

Mechanisms of Biological Removal of Phosphorus

❖Phosphorus removal is done by ***adsorption to sediment, plant uptake and bacteria uptake.***

❖ ***Biological phosphorous removal process relies on enhancing the ability of microorganisms to uptake more phosphorous into their cells.***

▪ These processes are often referred to as ***Enhanced Biological Phosphorous Removal (EBPR)***.

❖ EBPR is depend on ***polyphosphate accumulating organisms (PAOs)*** that are able to accumulate polyphosphate, by storing more phosphorus than they need for growth, i.e. ***"luxury uptake".*** *One of the organisms used as PAO is* ***bacteria.***

▪ ***EBPR*** basically consists of consequent **anaerobic and aerobic zone** for the processes.

❖ The ability to store the polyphosphate depends on the **availability of volatile fatty acids (VFAs),** which are generated through the *hydrolysis and fermentation by non-polyphosphate accumulating organisms* of the complex organic matter present in the wastewater or introduced externally.

➢ ***PAO contain three internal cell storage products*** relevant for excess **P** removal:

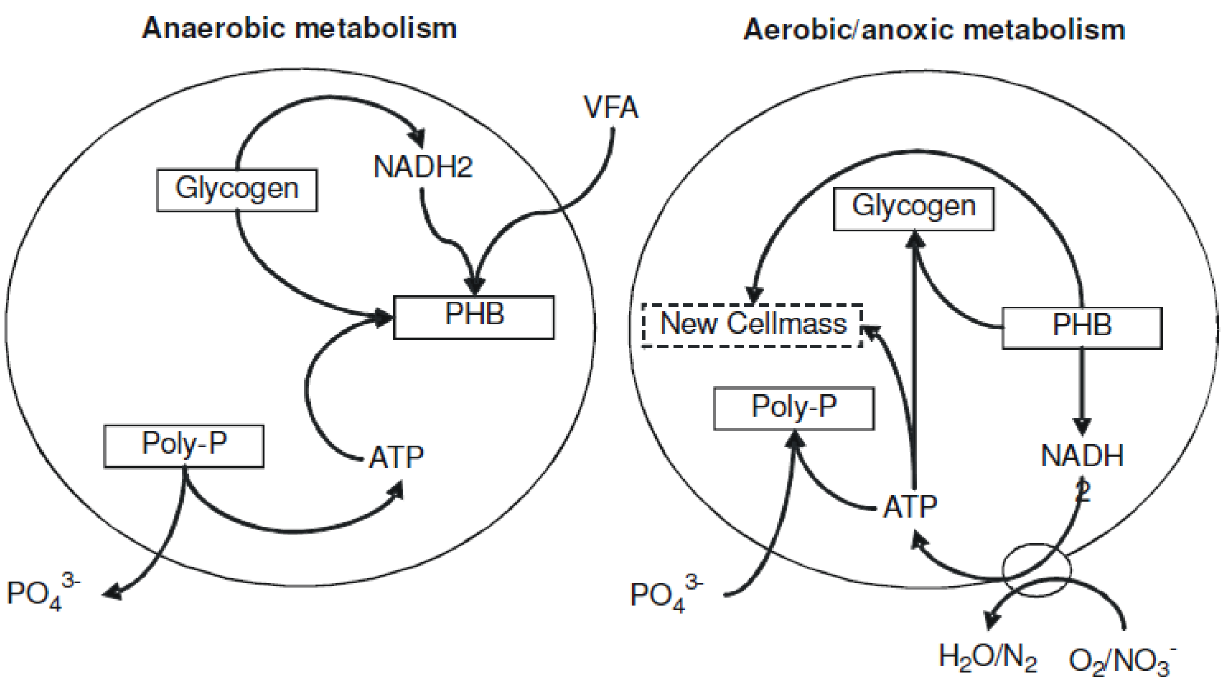
(1) Polyphosphate (2) Polyhydroxy-alkanoates (PHB) and (3) Glycogen (see figure in the next slide)

a) ***Under anaerobic conditions,***

* Volatile fatty acids are taken up from the liquid phase and stored as PHB.
* An important intermediate in this process is NADH2, an energy carrier released during the formation of PHB from glycogen.
* The energy required comes from the hydrolysis of polyphosphate and the subsequent formation of ATP;

b) Under anoxic or aerobic conditions,

* The stored PHB will be oxidised to CO2, releasing energy in the form of NADH.
* NADH will be used to create ATP, which in turn will allow the PAO organism to grow and restock with polyphosphate and glycogen.
* The main difference between the metabolism of PAO under anoxic and aerobic conditions is the ratio between ATP formed/NADH2 used: this ratio is about 40% lower under anoxic conditions.
* This explains the lower growth rate observed under anoxic conditions and also applies to normal heterotrophic organisms.



Heterotrophic microbes like bacteria that require **organic compounds** for the supply of **carbon and energy**.

Figure that shows Metabolism of PAO under anaerobic and anoxic conditions, (Smolders et al 1994).

❖ Molecular techniques have identified a group of Rhodocyclus- related bacteria, named "*Candidatus Accumulibacter phosphatis*", as PAOs.

❖ Bacteria with enhanced aerobic phosphorus uptake ability are includes *Acinetobacter calcoaceticus*, *Acinetobacter iwoffi* and *Aeromonas hydrophila*.

✓ Some bacterial strains have been found to take up enhanced amounts of phosphorus under solely aerobic conditions.

✓ The possibility to by-pass the anaerobic step is advantageous from a process design point of view. ***But, detail research works should be done to verify the possibility.***

Microalgae

➢ Many studies demonstrated that **microalgae** have a great potential for the removal of nitrogen and phosphorus. (Detail will be described in the next chapter)

➢ The main mechanisms in algal nutrient removal from wastewater include uptake into the cell and stripping ammonia through elevated pH (Hoffman, 1998; Bich et al., 1999).

➢ ***The advantages of using algae for this purpose include:***

* the low cost of the operation,
* the possibility of recycling assimilated nitrogen and phosphorus into algae biomass as a fertilizer and biofuel avoiding a sludge handling problem, and
* the discharge of oxygenated effluent into the water body.

Wastewater Treatment Technology Selection Issues

❖Selection of the best treatment option for remediation of a specific industrial wastewater is a highly complex task.

❖ The choice of one or more processes to be combined in a certain situation should cover both the ***technical, economical, societal (quality standards to be met)a***nd others.

➢ ***Some of the main factors considered in the decision on the wastewater treatment technologies are:***

- The quality/composition of the original wastewater
- Removal of parent contaminants, Performance, Coordination with local climate
- Conventional treatment options, resistance to hydraulic and organic load shocks
- Availability and selection of land, Treatment flexibility, Flexibility in operation
- The facility decontamination capacity, Coordination with local facilities
- Final wastewater treatment system efficiency,
- Economic studies,
- Life Cycle Assessment to determine environmental compatibility of the wastewater treatment technology
- Potential use of treated water.

❖ ***From the practical point of view, some additional points should be considered:***

– ***Know the Regulations –*** treatment standards & design requirements

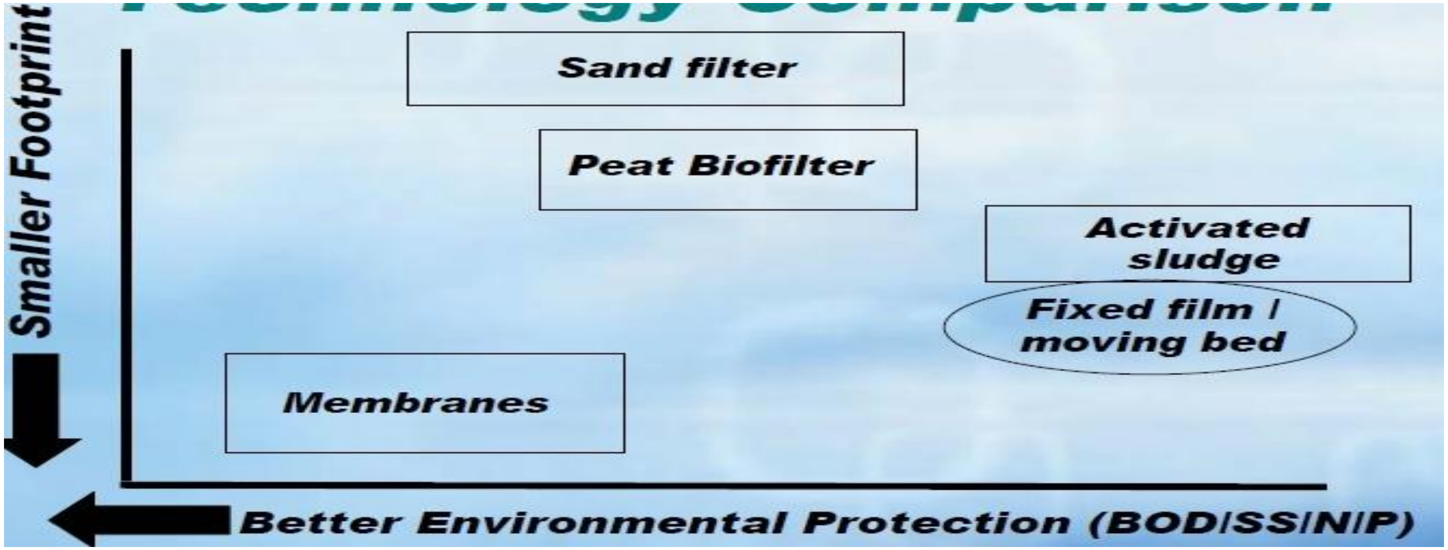
– ***Evaluate flows & loads and peaking factors carefully –*** the size & cost of the plant will directly relate to flows, loads and peaks.

– ***Consider an equalization tank to optimize treatment –***need to design for the peak flow.

– ***Evaluate the whole life cost of each viable option***

– ***Consider the complexity of technology and who will manage***
– ***Volume of sludge produced (daily, monthly and annually)***
– ***Footprint, Visual impact, Noise & Odor***
– ***Phased construction***

Technology Comparison



❖ The **set of indicators** (refer table in the next slide) viz., ***reliability, durability and flexibility***, ***quantifies the robustness of the technology***, and thus, these three 'qualitative' indicators are grouped under one criterion called 'robustness of the system.'

❖ The ***sustainability of the wastewater treatment*** alternative has been quantified using four indicators, viz., ***acceptability/simplicity, participation/responsibility, replicability and promotion of sustainable behavior***, grouped under the criterion 'sustainability.'

❖ Criteria with respective indicators and scores used for selection of appropriate wastewater treatment technology.

Sr no.	Criteria	Indicator	ASP	SBR	UASB-FAL	CWs
1	Global warming[a]	Global warming potential (kg CO_2-Eq/p.e.-year)	18.20	31.97	7.67	−3.86
2	Eutrophication[a]	Eutrophication potential (kg PO_4^{3-}-Eq/p.e.-year)	3.76	1.38	5.85	3.40
3	Life cycle costs[b]	Net present worth (Rs. Lakh/MLD)	137	127	103	242
4	Land requirement	Land requirement (m^2/MLD)	1400	353	1123	8500
5	Manpower requirement for operation	Number (for operation of medium scale plant)	10	6	14	4
6	Robustness of the system[c]	Reliability	80	80	60	40
		Durability	80	60	60	40
		Flexibility	80	60	40	30
7	Sustainability[c]	Acceptability	30	30	50	90
		Participation	30	30	50	80
		Replicability	40	40	40	80
		Promotion of sustainable behavior	40	40	60	90

a Estimated using ISO 14040: 1997 as described in Guinee et al. (2001) using CML 2 baseline 2000 methodology, only operation phase is considered for the analysis.

b Life cycle costs estimated as per the present worth method prescribed in IS 13174, (1994).

c These indicators do not have units as they are qualitative in nature. To quantify these indicators, a cardinal scale of 0 and 100 is used with 0 being the worst score and 100 the best score.

Conventional and Recent Developments in Bioreactor Configurations for Wastewater Treatment

- Waste treatment is considered in a broad context including concentration by bioaccumulation, degradation to substances with reduced environmental impact and upgrading to such useful products as feeds, foods and fuels.
- Undeniably, generation of gaseous, liquid and solid wastes is an unavoidable consequence of industrial, agricultural and domestic activities.
- ***Reduced waste generation***, ***improved treatment*** *and* ***utilization of wastes*** *will remain an* ***essential component of an overall strategy for maintenance of environmental quality.***

❖ Treating the wastewater by biological using stabilization ponds, aerated lagoons and activated sludge processes have positive and negative effects.

- Successful in lowering the chemical (**COD**) and biological oxygen demands (**BOD**), but their applicability is limited by a great number of problems.
- These biologically treated effluents still contain significant amounts of colored compounds, microorganisms, recalcitrant organics and a minor amount of biodegradable organics, as well as suspended solids.
- Biological treatment does not significantly reduce the inorganic content in the effluent and desalting is sometimes needed before reuse of the effluents in the manufacturing processes.
- These methods are much less efficient in toxicity reduction of the effluent.
- Given the limitations of the current biological wastewater treatment, ***there is an increasing interest to develop a more effective treatment approach*** to reduce the impacts of effluents on the environment.

- Among the most ***serious contaminants*** (both in terms of their impact and resistance to treatment) are toxic organic compounds, particularly ***aromatic and halogenated compounds*** and the subset of these known as ***xenobiotics*.**
- ***Xenobiotic and toxic compounds*** are materials that are invariably man-made and are foreign to nature in the sense that they have been present in the ecosphere for relatively short periods of time and therefore ***efficient biodegradation pathways have not had adequate time to evolve*.**

❑ ***In the biological treatment of xenobiotic compounds, the most significant challenge is substrate delivery.***

- Addition of the substrate at too high concentration will inhibit or even kill the organisms, whereas substrate addition at too low rate will cause the cells to starve and result in a sub-optimal process performance.
- Consequently, the biological treatment of these materials is particularly challenging owing to the inhibition and/or toxicity of these compounds when they serve as microbial substrates.

- In response to the inherent toxic nature of xenobiotic compounds, a process, rather than a microbial approach, has led to the development of an extremely promising technology for the treatment of toxic organic contaminants. This process is based on the use of **Bioreactor.**

Conventional Wastewater Treatment

➢ ***In conventional wastewater treatment facilities,***

1) The ***objective of preliminary treatment*** is the removal of coarse solids and other large materials often found in raw wastewater.

   * Removal of these materials is necessary to enhance the operation and maintenance of subsequent treatment units.
   * Typically include coarse screening, grit removal and , in some cases, comminution of large objects.
   * In grit chambers, the velocity of water through the chamber is maintained sufficiently high, or air is used, so as to prevent the settling of most organic solids.
   * Grit removal is not included as a preliminary treatment in most small wastewater treatment plants.
   * Flow measurement devices, often standing-wave flumes, are always included at the preliminary treatment stage.

2) The ***objective of primary treatment*** is the removal of settable organic and inorganic solids by sedimentation, and by removal of materials that will float (scum) by skimming.

➢ In conventional wastewater treatment facilities, primary treatment includes:

a) Screening and comminuting for removal of large solids
b) Grit removal
c) Sedimentation

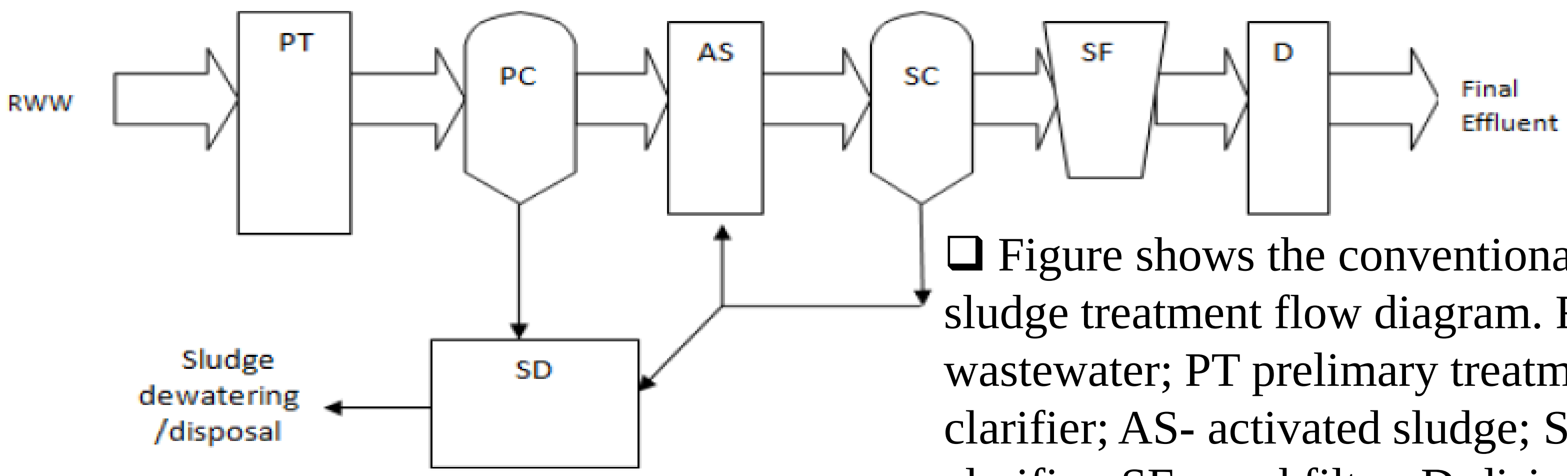
Conventional primary treatment is effective in removing of:

a) Particulate matter larger than about 50 μm from wastewater. In general, about 50% of the suspended solids and 25to 50% of the BOD_5 are removed from untreated wastewater.

b) Nutrients, hydrophobic constituents, metals, and microorganisms that are associated with particulates in wastewater. About 10 to 20% of the organic nitrogen and about 10% of the phosphorous are removed.

- Primary sedimentation tanks or clarifiers may be round or rectangular basins, typically 3 to 5 m deep, with hydraulic retention time between 2 and 3 hours.

- Settled solids (primary sludge) are normally removed from the bottom of tanks by sludge rakes that scrape the sludge to a central well from which it is pumped to sludge processing units.
- Scum is swept across the tank surface by water jets or mechanical means from which it is also pumped to sludge processing units.



- Figure shows the conventional activated sludge treatment flow diagram. RWW- Raw wastewater; PT prelimary treatment; PC- primary clarifier; AS- activated sludge; SC- secondary clarifier; SF- sand filter; D-disinfection; SD- sludge digestion.

- The removal efficiency of primary treatment processes can be increased by incorporating coagulation/flocculation upstream of gravity sedimentation and /or by using filtration downstream of gravity sedimentation.

- For most wastewater reuse applications, primary treatment alone does not provide adequate treatment to meet water quality objectives.

3) The objective of **secondary treatment** is the further treatment of the effluent from primary treatment ***to remove the residual and suspended solids***.

- ✓ In most cases, secondary treatment follows primary treatment and involves the ***removal of biodegradable dissolved and colloidal organic matter using aerobic biological treatment processes***.

- Aerobic biological treatment is performed in the presence of oxygen by aerobic microorganisms (principally bacteria) that metabolize the organic matter in the wastewater, thereby ***producing more microorganisms and inorganic end-product (principally CO2, NH3, and H2O).***

- Secondary treatment systems include an array of biological treatment processes coupled with solid/liquid separation.

- Biological processes are engineered to provide effective microbiological metabolism of organic substrates dissolved or suspended in wastewater. The microbial biomass interacts with wastewater using a suspended growth or a fixed film process.

Several aerobic biological processes are used for secondary treatment differing primarily in:

a) Manner in which oxygen is supplied to microorganisms, and

b) Rate at which organisms metabolize the organic matter, which can be

* High rate biological processes
* Low rate biological processes

Common high-rate processes include:

1. Activated sludge processes
2. Trickling filters or biofilters
3. Oxidation ditches
4. Rotating biological contractors

Common low-rate processes include:

1. Waste Stabilization Ponds
2. Overland Treatment of Wastewater
3. Macrophyte Treatment
4. Nutrient Film Technique

High rate biological processes are characterized:

1. By relatively small reactor volumes and high concentration of microorganisms compared with low rate processes.
2. The growth rate of new organisms is much greater in high rate systems because of the well controlled environment

- A combination of two of these processes in series (e.g., biofilter followed by activated sludge) is sometimes used to treat municipal wastewater containing a high concentration of organic material from industrial sources.

- In the activated sludge process, the dispersed-growth reactor is an aeration tank or basin containing a suspension of wastewater and microorganisms, the mixed liquor.
  - The contents of the aeration tank are mixed vigorously by aeration device (submerged diffusers that release compressed air or mechanical surface aerators that introduce air by agitating the liquid surface) which also supply oxygen to the biological suspension.

- Hydraulic retention time in the aeration tanks usually ranges from 3 to 8 hours but can be higher with high BOD_5 wastewaters.

- Following the aeration step, the microorganisms are separated from the liquid by sedimentation and the clarified liquid is secondary effluent.

- A trickling filters or biofilter consist of a basin or tower filled with support media such as stones, plastic shapes, or wooden slats.
  - Wastewater is applied intermittently, or sometimes continuously, over the media.
  - Microorganisms become attached to the media and form a biological layer or fixed film.
  - The thickness of the biofilm increases as new organisms grow, and periodically a portion of the film slough off the media and it is separated from the liquid in a secondary clarifier and discharged to sludge processing.
  - Clarified liquid from the secondary clarifier is the secondary effluent and a portion is often recycled to the biofilter to improve hydraulic distribution of the wastewater over the filter.

Bioreactor Configurations for Wastewater Treatment

WHAT ARE BIOREACTORS?

A bioreactor may refer to any manufactured or engineered device or system that supports a biologically active environment.

A bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic

The Role of bioreactors in biotechnology:
To reach its' necessary goals, the biotechnological process has usually 3 major stages:
1. Preparation of nutrient media for the cultivated microorganism and the cultivation process;

2. The course of the microorganism reproduction process in bioreactors (called also fermenters) or in other equipment;

3. Obtaining of the final product or substance from the cultivated medium. This stage includes operations such as separation, purification and other technologies, which are connected with obtaining the commodity form.

TYPES AND CLASSIFICATIONS OF BIOREACTORS

Bioreactors are generally classified into two broad groups;

1. SUSPENDED GROWTH BIOREACTORS;

The reactors use microbial metabolism under aerobic, anaerobic, or sequential anaerobic/aerobic conditions to biosorb organic compounds and biodegrade them to innocuous residuals. The microbial activity in the systems produces biomass that is removed by gravity sedimentation, with a portion of the settled biomass recycled to maintain a desired mixed liquor suspended solids concentration in the bioreactor. E.g Batch reactors, CSTR'S, Plug-flow reactors etc

2. BIOFILM BIOREACTORS:

In biofilm reactors most of the microorganisms are attached to a surface, and in this manner kept within the reactor. Biofilm is also used regularly for wastewater treatment, and the bacteria can either absorb or break down toxic substances in the water. The different kinds of biofilm reactors include membrane, fluidized bed, packed bed, airlift, and upflow anaerobic sludge blanket reactors.

MASS BALANCES

A mass balance (also called a material balance) is an application of conservation of mass to the analysis of physical systems.
By accounting for material entering and leaving a system, mass flows can be identified which might have been unknown, or difficult to measure without this technique.

The mass balance is the key to design and analysis of microbial processes. A mass balance is provided by a balanced stoichiometric chemical equation.

EXAMPLE:
0.0333C6H5COO- + 0.12NO3- + 0.002NH4+ + 0.12H+ ------
0.02 C5H7O2N + 0.06N2 +0.12CO2 +0.0133HCO3 + 0.1067H2O

0.033C6H5COO- = BENZOATE TO BE CONSUMED BY MICROBES
0.12NO3- = ELECTRON ACCEPTOR
0.02NH4+ = AMMONIUM TO BE CONSUMED BY MICROBES AS NUTRIENT

0.O2C5H702N = BIOMASS PRODUCED AFTER DEGRADATION BY MICROBES
0.06N2, 0.12C02, 0.0133HCO3, 0.1067H2O =(NIRTROGEN,CARBON DIOXIDE , CARBONATE AND WATER PRODUCED DUE TO THE DEGRADATION

A very important aspect of mass balances is that each component must have their own mass balance.
Components may include, oxygen, electron acceptor, TOC, COD,biomass, ammonium and nitrate and macro nutrients.

RATE OF MASS ACCUMULATION IN CONTROL VOLUME = RATE(S) MASS IN - RATE(S) OF MASS OUT + RATE OF MASS GENERATION

MASS BALANCE FOR BATCH REACTORS

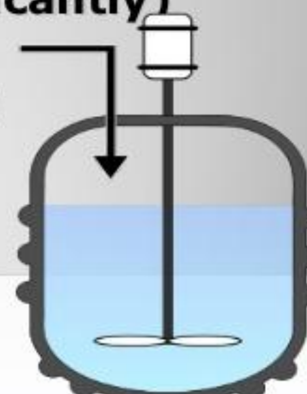
The batch reactor is assumed well stirred, and also let the entire reactor contents be the reactor volume element.
Hence,

d(VC)/dt = Q in . C in - Q out . C out + R.V

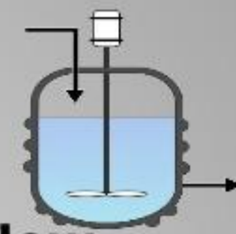
Where d(VC)/dt = Rate of mass accumulation in control volume
Q in = flow rate into the system
Q out = flow rate out of the system
C = Concentration of stream/substrate
R = Rate of reaction
V = Volume of the stream/ substrate

The inflow and outflow stream rates are zero, Q in – Q out = 0
Hence , we have
d(VC)/dt = R.V (if reactant volume changes significantly)
or
d(C)/dt = R (if reactant volume remains constant)

R= k. C, where k = rate constant, c = concentration



MASS BALANCE FOR THE CONTINUOUS STIRED TANK BIOREACTOR



Writing the material balance for this reactor gives,

d(VC)/dt = Q in.Cin - Q out.Cout + R.V

If the reactor volume is constant and the volumetric flow rates of the inflow and outflow streams are the same, then

d(C)/dt = 1/Ŧ (C in - C out + R)

This parameter Ŧ = V/Q in it is called the mean residence time of the CSTR.

We refer to this balance as the constant-density case. It is often a good approximation for liquid-phase reactions.

for steady state:

The steady state of the CSTR is described by setting the time derivative in the expression d(VC)/dt = 0

0 = Q in . C in - Q out . C out + R.V

Conversion of reactant x is defined for a steady-state CSTR as follows:

X = (Q in. C in - Q out. C out) / Q in .C in

CONCLUSIONS

Bioconversion of wastes to harmless substances or higher value products already has a significant role in environmental pollution control and improved resource utilization. Both in-situ and bioreactor based treatment processes are experiencing rapid development and increasing deployment in practical applications.

BIOREACTOR SYSTEM SELECTION CRITERIA

- The ***ideal bioreactor configuration*** for treatment of organic or inorganic contaminants present in industrial wastewaters or other aqueous streams include the following:

1) The ***characteristics of the contaminants*** *in question with respect to such factors as* ***biotreatability*** (e.g., readily biodegradable by large consortium of bacteria, slowly biodegradable by selected bacteria), ***soluble versus colloidal contaminants***, ***presence of particulates***, ***contaminant volatility*** and the ***sorbable characteristics of the contaminants*** (E.g., adsorbable on granular activated carbon (GAC)).
2) ***The ability to design and operate the bioreactor under the required and/or optimal biological process conditions for treatment of the contaminants*** (e.g., aerobic, anoxic or anaerobic conditions dictated by the required or ideal electron acceptor, mesophilic or thermophilic temperature conditions, complete-mix or plug-flow hydraulic conditions dictated by the relationship between contaminant concentration and rate of biotreatment).
3) ***The ability to control and regulate the biomass inventory in the bioreactor, maximizing the active fraction and preventing loss due to variable or inhibitory feed conditions*** or ***an adverse change in process operating conditions.***
4) The ***mechanical simplicity of the bioreactor configuration, capital and operating costs, ease of operation and maintenance requirements for the system.***

Key Issues in Bioreactor Design and Operation

❖ ***Although the majority of fundamental bioreactor engineering and design issues are similar***, ***maintaining the desired biological activity and eliminating or minimizing undesired activities often presents a greater challenge*** than traditional chemical reactors typically require.

❖ ***The goal of an effective bioreactor is to control, contain and positively influence the biological reaction.***

❖ ***To accomplish this, the chemical engineer must take into consideration two areas.***

A) The ***suitable reactor parameters*** for the desired biological, chemical and physical (macrokinetic) system.

▪ The macrokinetic system includes microbial growth and metabolite production.

▪ Microbes can include bacteria, yeast, fungi as well as other biological materials.

B) Bioreactor design involves ***the bioreaction parameters***, including:

- controlled temperature
- optimum pH
- sufficient substrate (usually a carbon source), such as sugars, proteins and fats
- water availability
- salts for nutrition
- vitamins
- oxygen (for aerobic processes)
- gas evolution
- product and byproduct removal and others.

Important factors need to be consider in designing Bioreactors

- Large volume and low value products like alcoholic beverages need simple fermenters and do not need aseptic condition.
- High value and low volume products require more elaborate system of operation and aseptic condition.
- The Designing of a Bioreactor also has to take into considerations the Unique Aspects of Biological Processes:

A: The concentrations of starting materials (substrates) and products in the reaction mixture are frequently low; both the substrates and the products may inhibit the process.

Cell growth, the structure of intracellular enzymes, and product formation depend on the nutritional needs of the cell (salts, oxygen) and on the maintenance of optimum biological conditions (temperature, concentration of reactants, and pH) within narrow limits.

B: Certain substances, inhibitors, effectors, precursors, metabolic products influence the rate and the mechanism of the reactions and intracellular regulation.

C: Microorganisms can metabolize unconventional or even contaminated raw materials (cellulose, molasses, mineral oil, starch, ores, wastewater, exhaust air, biogenic waste), a process which is frequently carried out in highly viscous media.

D: In contrast to isolated enzymes or chemical catalysts, mo's adapt the structure and activity of their enzymes to the process conditions, whereby selectivity and productivity can change.

Mutations of the microorganisms can occur under sub optimal biological conditions.

E: Microorganisms are frequently sensitive to strong shear stress and to thermal and chemical influences.

F: Reactions generally occur in gas-liquid -solid systems, the liquid phase usually being aqueous.

G: Continuous bioreactors often exhibit complicated dynamic behavior.

H: The microbial mass can increase as biochemical conversion progresses.

Effects such as growth on the walls, flocculation, or autolysis of microorganisms can occur during the reaction.

Requirements of Bioreactors

There is no universal bioreactor.

The general requirements of the bioreactor are as follows:

- The design and construction of bioreactors must keep sterility from the start point to end of the process.
- Optimal mixing with low, uniform shear.
- Adequate mass transfer, oxygen.
- Clearly defined flow conditions.
- Feeding substrate with prevention of under or overdosing.
- Suspension of solids.
- Gentle heat transfer.
- Compliance with design requirements such as: ability to be sterilized; simple construction; simple measuring, control, regulating techniques; scale-up; flexibility; long term stability; compatibility with up-downstream processes; antifoaming measures.

Design Calculations for Bioreactor

- **Factors to be considered**

– Inlet / outlet pumping stations
– Trash tank with effluent filter
– Equalization tank
– Inlet screens
– Chemical dosing for Phosphorus reduction, Alkalinity correction, Carbon addition
– Call-out alarm or remote monitoring facility
– Stand-by generator, Standby equipment philosophy - blowers
– Walkways & lifting equipment
– Membrane cassettes to be light and easy to remove & handle
– Reuse systems will require permeate storage
– Compatible with standard on-site tanks
– Sludge holding tanks
– Covers & odor control
– Seed sludge to accelerate commissioning

Engineering & Practical Design Considerations

- ***For small flows look for reduced complexity & reduced operational input***

– Flat plate generally better than hollow fiber
– No back pulsing or chemicals
– Gravity design for community systems or permeate pumps for residential
– Long periods between chemical cleans

- ***Design***

– Always consider peak flows
– Flux rate – compare at the same temperature
– Screening requirements
– Ultra or micro filtration - pathogen removal
– Operation cost - power
– Life of membrane material

Recent Developments in Bioreactor Configurations for Wastewater Treatment

❖ *The volumetric efficiency of any biological treatment process is dependent on maintaining a high active biomass concentration in the reactor.*

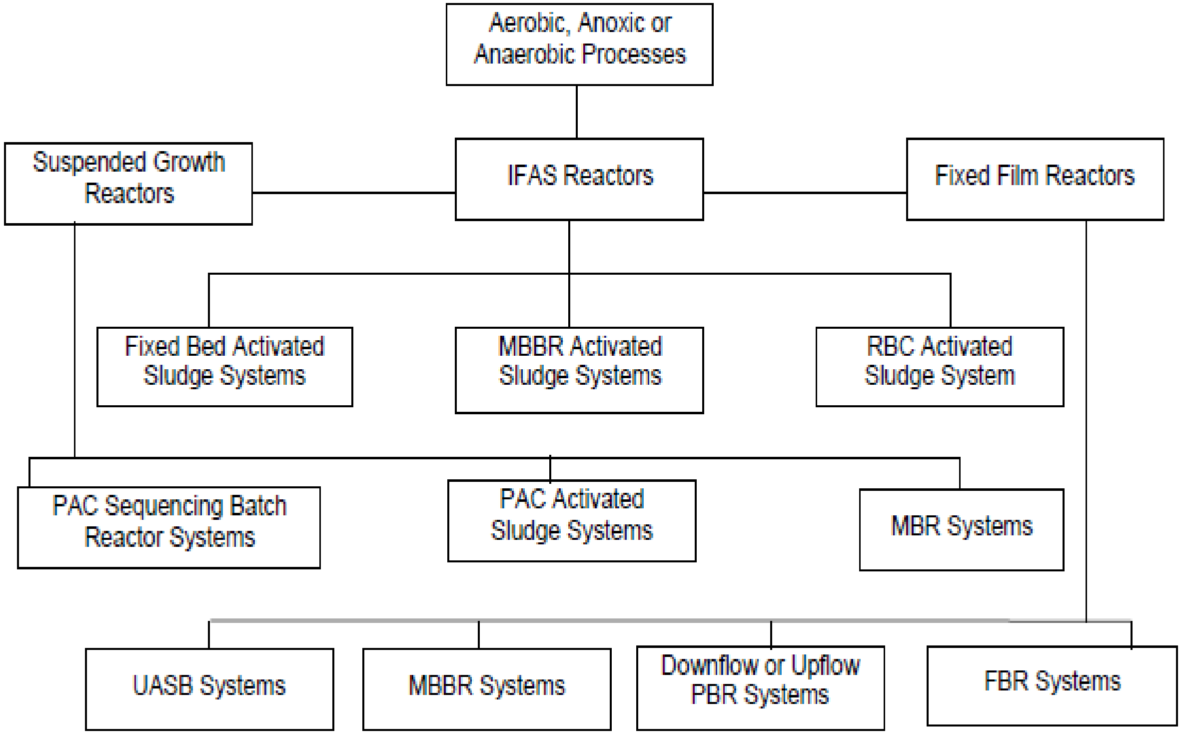
❖ ***Biological reactors can be classified according to the nature of their growth.***

a) Those in which the active biomass is suspended as free organisms or microbial aggregates can be classified as suspended growth reactors, whereas those in which growth occurs on or within a solid media can be termed supported growth or fixed film reactors.

b) Reactors involving the use of a fixed film media located in a suspended growth reactor are termed hybrid reactors or integrated fixed film activated sludge (IFAS) reactors.

➢ The major bioreactor configurations commercially available and capable of operation under aerobic, anoxic or anaerobic conditions at higher volumetric loading rates, are categorized in the following figure.

▪ **IFAS** = integrated fixed film activated sludge, **MBBR** = moving bed biofilm reactor, **RBC** = rotating biological contactor, **PAC** = powdered activated carbon, **MBR =** membrane biological reactor, **UASB =** upflow anaerobic sludge blanket, **PBR =** packed bed reactor and **FBR =** fluidized bed reactor.



Membrane Bioreactor Configuration

- Employ ***biological reactor and membrane filtration*** as a unified system for the secondary treatment of wastewater
- *The membrane bioreactors can be broadly defined as systems integrating biological degradation of waste products with membrane filtration.*
- ***Membranes perform the separation of the final effluent from the biomass through filtration.***
- ***Filtration takes place by the application of a pressure gradient.***
- ***Combining membrane technology with biological reactors*** for the treatment of wastewaters has ***led to the development of three generic membrane bioreactors*** (MBRs): ***for separation and retention of solids; for bubbleless aeration within the bioreactor***, and ***for extraction of priority organic pollutants from industrial wastewaters.***

➢ Studies showed the **membrane bioreactors (MBRs)** have proven quite effective in removing both organic and inorganic contaminants as well as biological entities from wastewater.

❖ ***The bioreactor and membrane module each have a specific function:***

(1) biological degradation of organic pollution is carried out in the bioreactor by adapted MOs

(2) separation of microorganisms from the treated wastewater is performed by the membrane

(3) the membranes constitute a physical barrier for all suspended solids and therefore causes not only recycling of the activated sludge to the bioreactor but also production of permeate free of suspended matter, bacteria and viruses.

❖ Each of the bioreactor configurations achieves efficiency by design of the reactor to maintain a high active biomass concentration.

- ❖ In the **MBR** configuration, a high concentration of biomass measured as volatile suspended solids (**VSS**) (i.e., normally greater than 10 g/l) is achieved by absolute retention of suspended matter with a particle size much smaller than that characterizing a bacterial cell (i.e., typically 0.3 to 0.5 microns) through use of a microfiltration or ultrafiltration membrane unit process.
- **VSS** concentration values in excess of 30 g/l have been maintained (Sutton et al., 2001) in external membrane based MBR systems (i.e., membranes located external to the bioreactor, and operated at high cross-flow velocity and transmembrane pressure or TMP values).
- ***The bioreactor VSS design concentration selected is normally dictated by membrane efficiency considerations (e.g., membrane permeability).***
- ➢ Industrial wastewaters, which can be difficult to treat without long solids retention times (SRTs), and wastewater operations where settling and clarification problems are regularly encountered, are typical applications of MBR technology.
- The use of membranes to separate solids and treated wastewater is the main difference betweens MBRs and conventional activated sludge systems for which the efficiency of the final clarification step depends mainly on the activated sludge settling properties.

- ➢ ***Treatment of wastewater by membrane technology is an established alternative, particularly in sensitive areas, water scarce regions, and in cases where wastewaters reuse and recycling is required (Drioli and Giorno, 2009).***
- ➢ Industries where the membrane bioreactor technology can be implemented include chemical, petrochemical, pharmaceutical, fine chemicals, cosmetics, diary, automotive, pulp and paper, landfill leachate, food, textiles, etc. (Wang and Menon, 2009).

Description of the MBR Process

❖ MBR systems essentially consist of a combination of membrane units responsible for physical separation, and biological reactor systems responsible for biodegradation of the waste compounds.

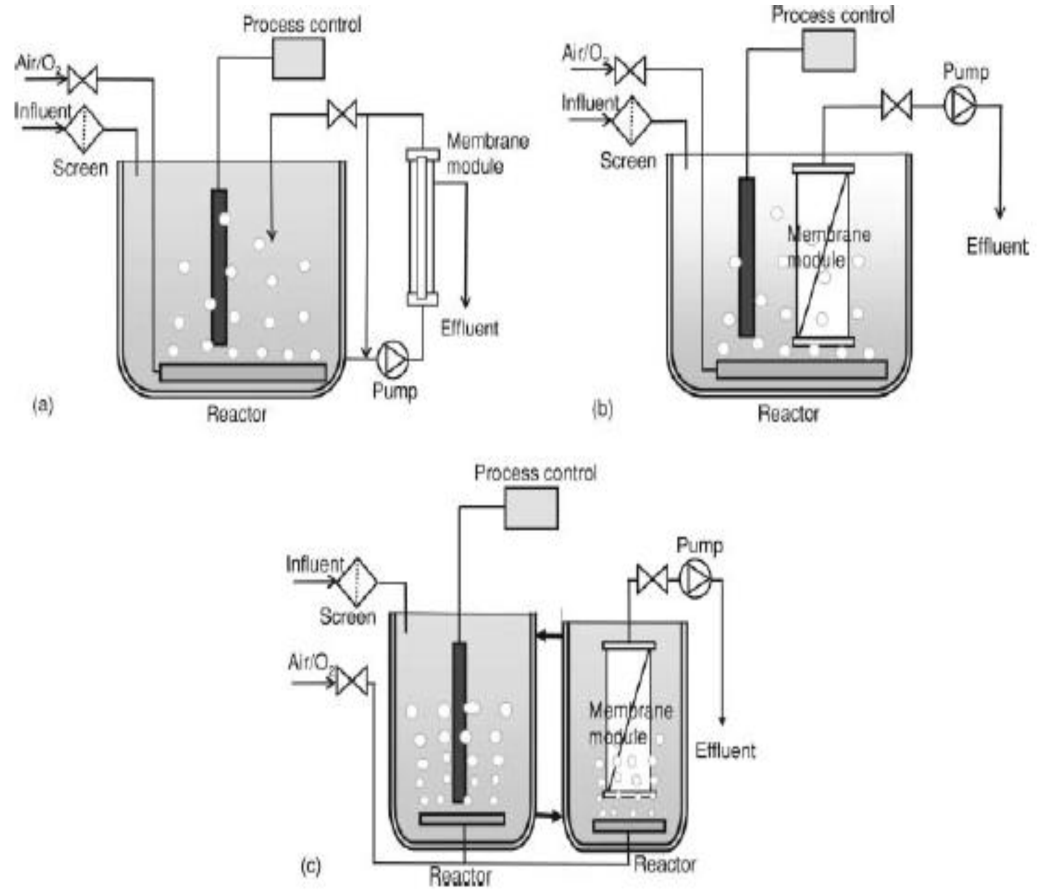
❖ ***These systems are implemented based on three main configurations:***

a) external/side-stream configuration (Figure 1a) and

b) submerged/immersed configuration (Figure 1b).

c) airlift configuration

❖ To date, both configurations (a and b) have been extensively employed for industrial WWT.



➢ The figure shows:

Membrane bioreactor configurations:

(a) external configuration,

(b) submerged configuration,

(c) airlift configuration.

❖ ***External configuration***, which involves the recirculation of the mixed liquor through a membrane module that is outside the bioreactor, usually **employs high cross-flow velocity** (CFV) along the membrane surface to provide membrane driving force and control membrane fouling.

- As a result, this configuration provides more direct hydrodynamic control of membrane fouling and offers the advantages of easier membrane replacement and high fluxes but at the expense of frequent cleaning and high energy consumption (2–12 kWh/m3 product).

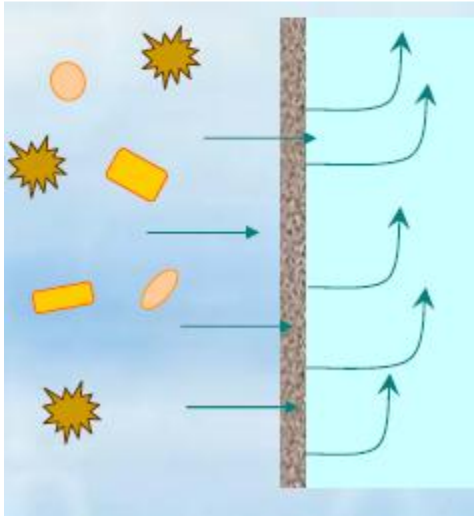
❖ ***Submerged configuration***, membrane modules are directly placed in the mixed liquor.

- The ***driving force across the membrane is achieved by pressurizing the bioreactor or creating negative pressure on the permeate side***.
- Some distinct advantages are their ***much lower energy consumption and less rigorous cleaning procedures***, as well as the ***operating conditions are much milder than in external MBR*** systems because of the lower tangential velocities.

❖ In recent years, many efforts have been made on the development of ***air-lift side-stream*** **MBRs** (Figure 1c).

- The concept applies the side-stream air-lift principle using a robust and reliable side-stream configuration while incorporating all the advantages of the low energy-consuming submerged systems.
- ***MBRs with this configuration have been tested for treatment of toilet wastewater, landfill leachate, pharmaceutical wastewater, and municipal wastewater.***

Membrane - Benefits



- High quality effluent
- Water reuse
- Small footprint
- Reduced sludge production
- Reduced cost of disinfection
- Cost effective for community size systems particularly where ammonia or total nitrogen standards are required
- Uprating existing systems

Parameter	MBR Effluent mg/l (%)	Conventional Effluent mg/l (%)
SS (mg/l)	<2 (99)	25 (73.2)
BOD_5 (mg/l)	4 (95.8)	19 (82.3)
COD (mg/l)	27 (88.5)	66 (77.2)
TN (mg/l)	9.2 (73.7)	15.9 (54.5)
TP (mg/l)	2.4 (36.1)	3.4 (8.6)

➢ ***Waste treatment is considered in a broad context including:***

* concentration by bioaccumulation,
* degradation to substances with reduced environmental impact and
* upgrading to such useful products as feeds, foods and fuels.