# Environmental Biotechnology

# Introduction

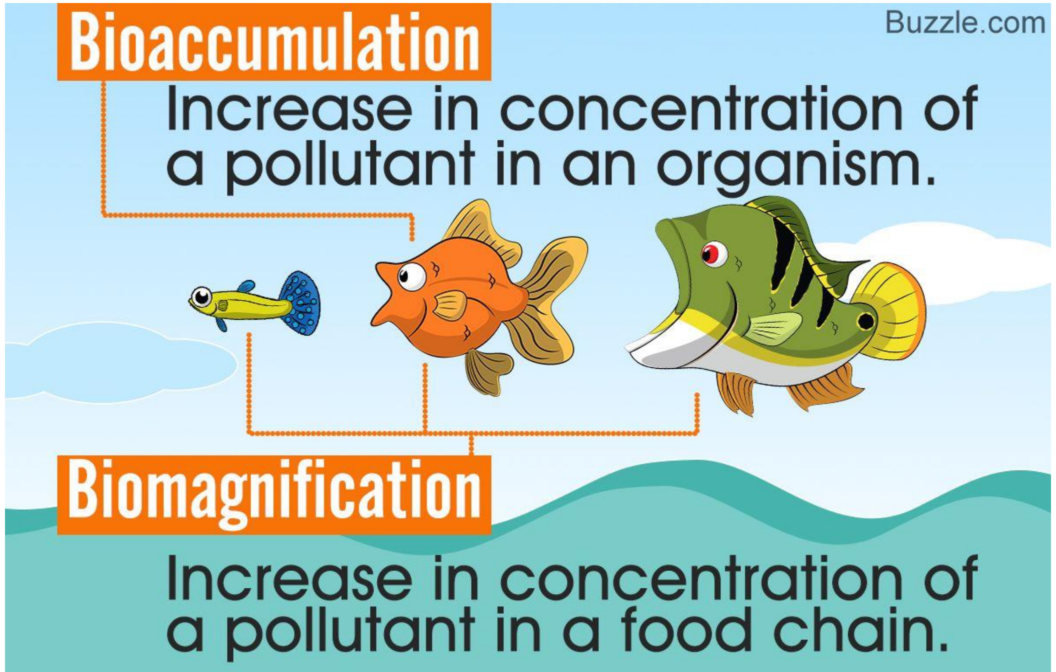
- Environmental Biotechnology is applied to and used to study the natural environment.
- Environmental biotechnology is **the branch of biotechnology that addresses environmental problems, such as the removal of pollution, renewable energy generation or biomass production, by exploiting biological processes**.
- It is concerned with the applications of biotechnology to solve the problems in ecosystem.
- It can be considered as a driving force for integrated environmental protection leading to sustainable development.
- The international society for environmental biotechnology defines it as “the development , use and regulation of biological systems for remediation of contaminated environment (land, air, water) and for environment friendly processes (green manufacturing technologies and sustainable development)”.
- Divided into five major types:
    - Bioaccumulation
    - Biodegradation
    - Bioremediation
    - Bioleaching
    - Biomethanation

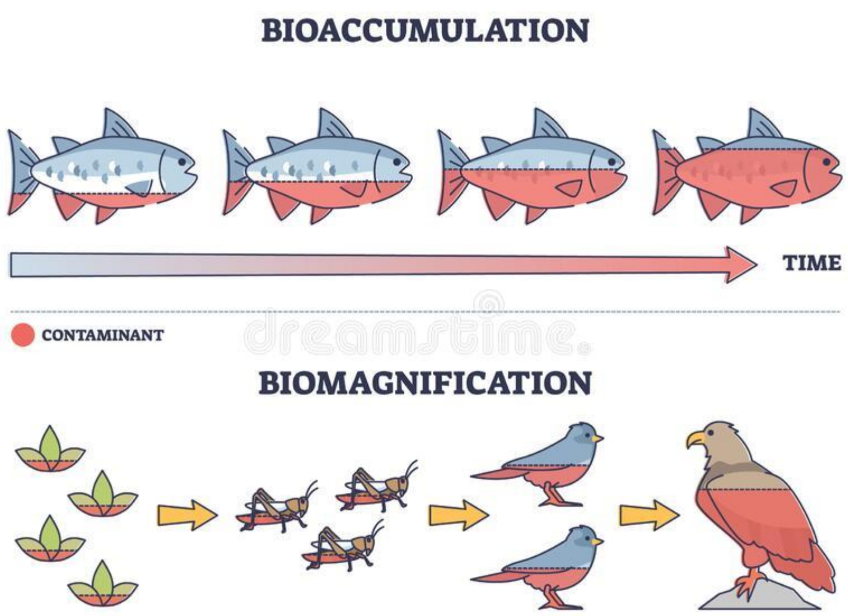
# 1. Bio-accumulation

- It refers to the accumulation of substances such as pesticides, or other organic chemicals in an organism.
- It occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost.
- Thus, the longer the biological half-life of the substance the greater the risk of poisoning, even if environmental levels of the toxin are not very high.
- For example, in fish it can be predicted by models.
- Biotransformation can strongly modify bioaccumulation of chemicals in an organism.
- Bioaccumulation is the result of three processes:
  - Uptake
  - Storage
  - Elimination

# <u>Bio-concentration and Bio-magnification</u>

- Bio-concentration refers to the uptake and accumulation of a substance from water alone. In contrast, bioaccumulation refers to the uptake from all sources combined (e.g. water, food, air, etc.)
- Biomagnification, also known as bio-amplification or biological magnification, is the increase in concentration of a substance that occurs in a food chain as a consequence of :
    - Persistence (cant be broken down by environmental processes)
    - Food chain energetic
    - Low (or non-existent) rate of internal degradation/excretion of the substance (often due to water insolubility).



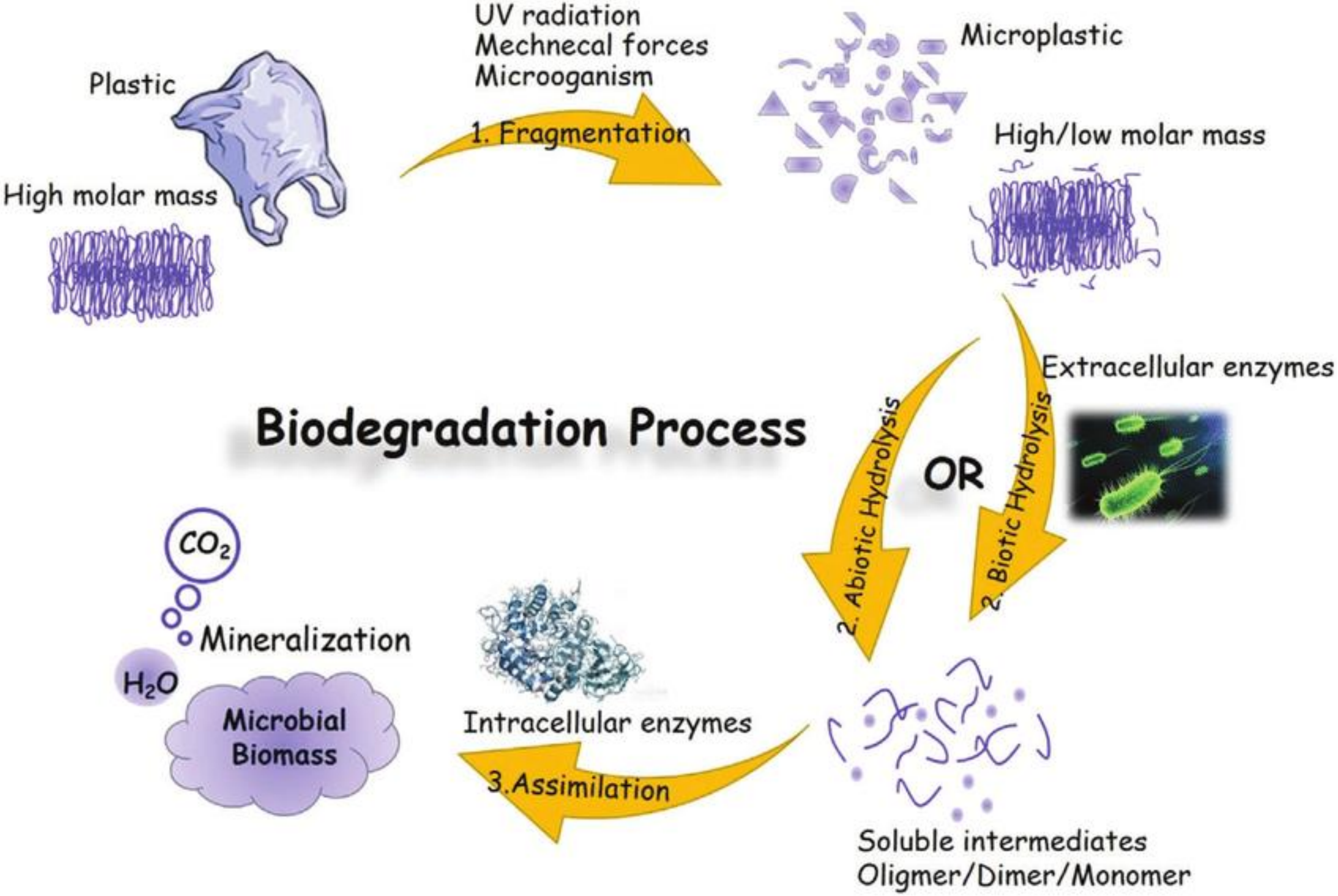



# 2. Biodegradation

- Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds.
- It the process by which organic substances are broken down into smaller compounds by living microbial organisms.
- The microbial organisms transform the substance through metabolic or enzymatic processes.
- Factors influencing microorganisms to use pollutants as substrates are temperature, pH, available nitrogen, phosphorus sources.
- It is based on two processes: growth and co-metabolism.

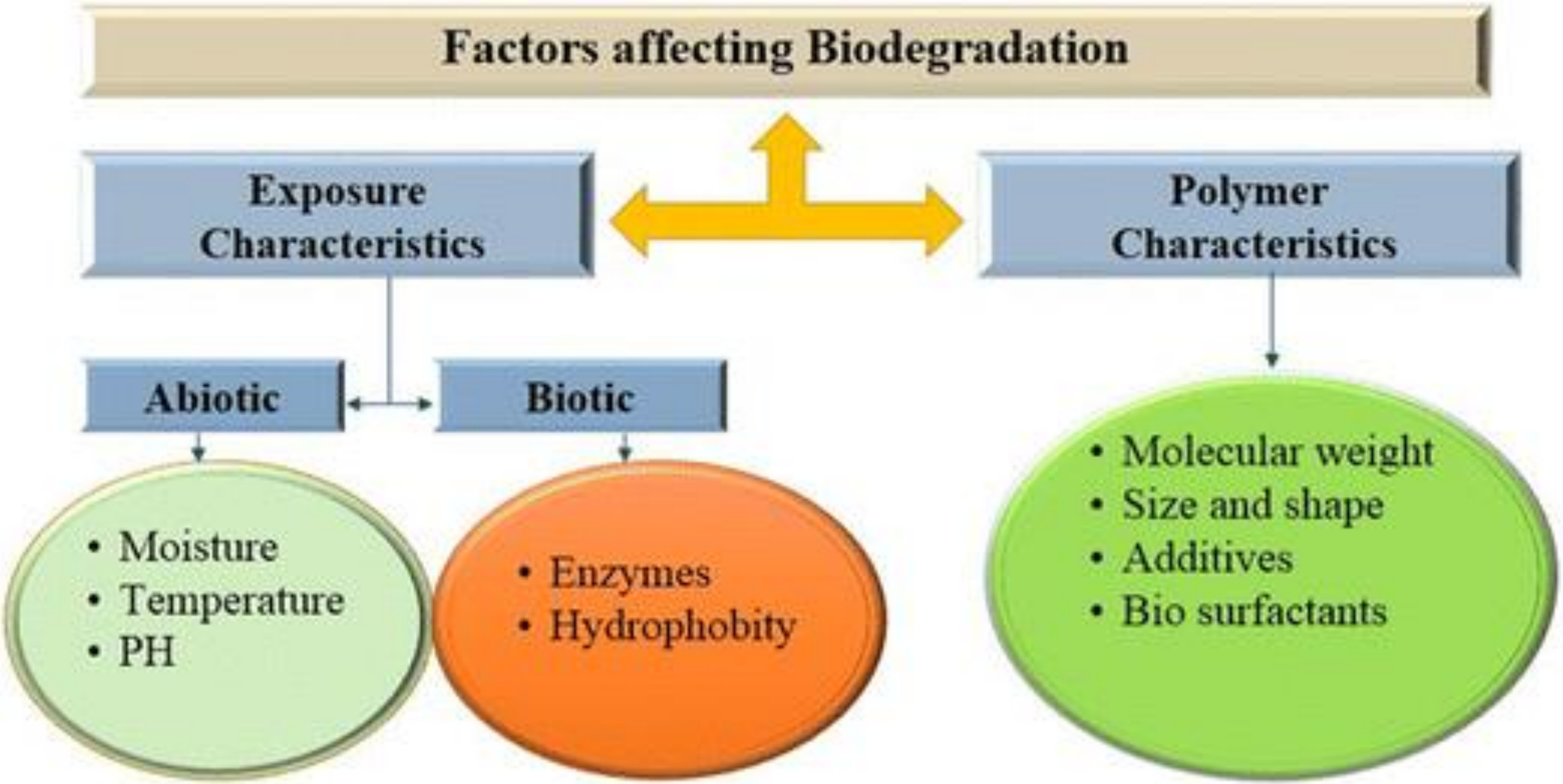
  In growth, an organic pollutant is used as sole source of carbon and energy. This process results in a complete degradation (mineralization) of organic pollutants.

  Co-metabolism is defined as the metabolism of an organic compound in the presence of a growth substrate that is used as the primary carbon and energy source. In other words, simultaneous degradation of two compounds, in which the degradation of the second compound (secondary substrate) depends on the presence of the first compound (primary substrate).
- Several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process. Algae and protozoa reports are scanty regarding their involvement in biodegradation
- Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms.


Plastic
High molar mass
UV radiation
Mechnecal forces
Microoganism
1. Fragmentation
Microplastic
High/low molar mass
Extracellular enzymes
Biodegradation Process
2. Abiotic Hydrolysis
OR
2. Biotic Hydrolysis
$CO_2$
Mineralization
$H_2O$
Microbial Biomass
Intracellular enzymes
3.Assimilation
Soluble intermediates
Oligmer/Dimer/Monomer

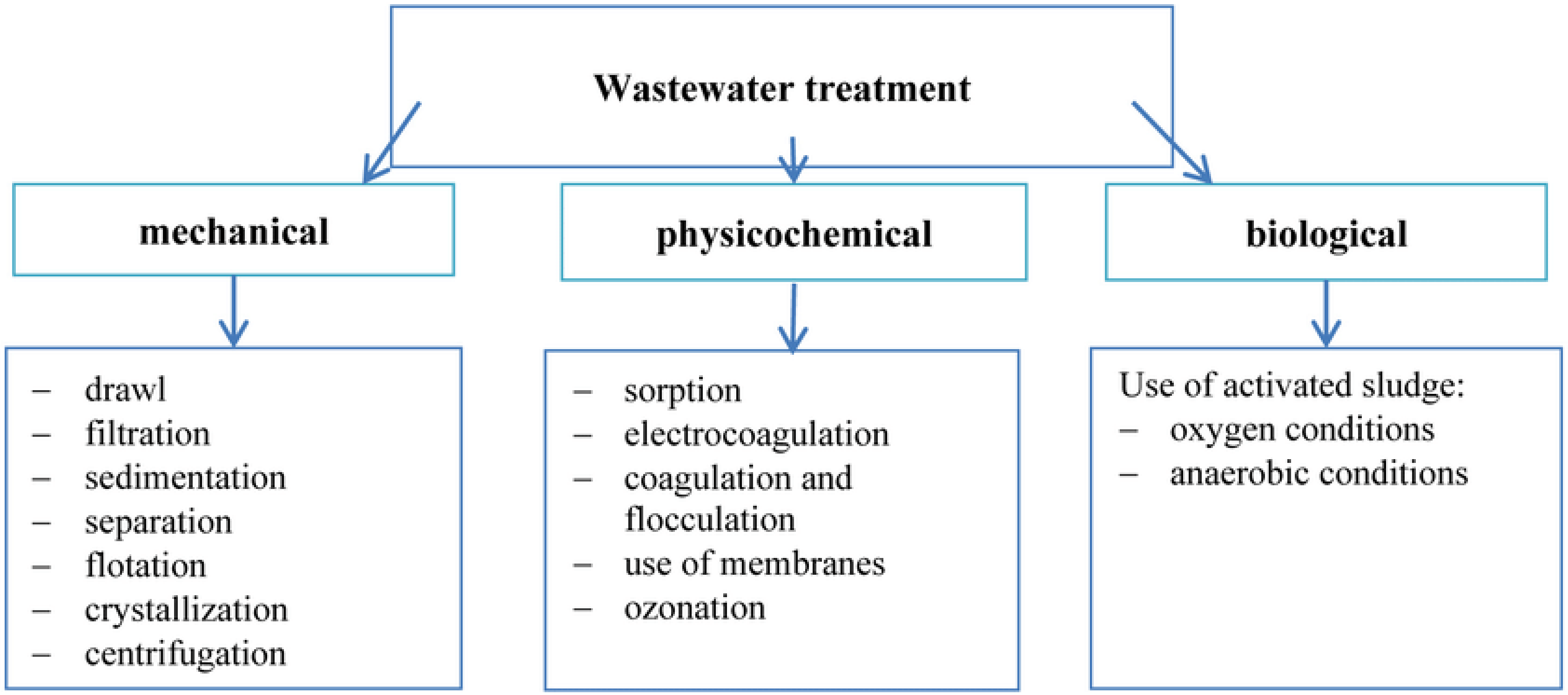
## Role of microorganisms in biodegradation of pollutants

- Some biodegradable pollutants are Hydrocarbons, PAHs, PCBs, pesticides, dyes, radionuclides and heavy metals etc.
- Biodegradation of hydrocarbons can occur under aerobic and anaerobic conditions, it is the case for the nitrate reducing bacterial strains *Pseudomonas* sp. and *Brevibacillus* sp. isolated from petroleum contaminated soil.
- Bacterial strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated, mainly from soil. These are usually gram negative bacteria, most of them belong to the genus *Pseudomonas*. The biodegradative pathways have also been reported in bacteria from the genera *Mycobacterium*, *Corynebacterium, Aeromonas, Rhodococcus* and *Bacillus*.
- Recent findings concerning pesticide degrading bacteria include the chlorpyrifos degrading bacterium *Providencia stuartii* isolated from agricultural soil and isolates *Bacillus*, *Staphylococcus* and *Stenotrophomonas* from cultivated and uncultivated soil able to degrade dichlorodiphenyltrichloroethane (DDT).
- several researchers have identified single bacterial strains that have very high efficacy for removal of azo dyes, it is the case of *Shewanella decolorations*

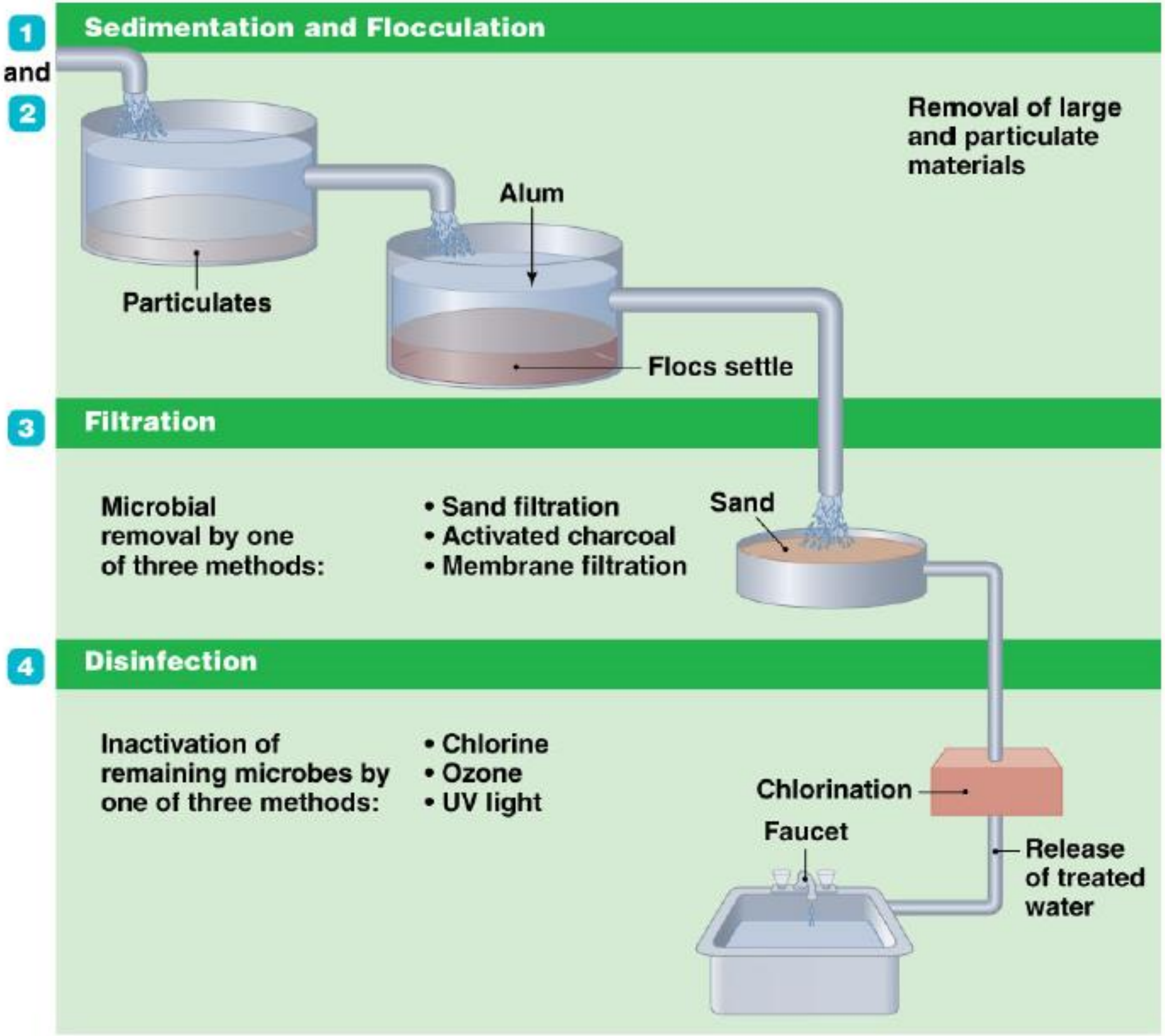

Factors affecting Biodegradation
Exposure Characteristics
Polymer Characteristics
Abiotic
Biotic
• Moisture
• Temperature
• PH
• Enzymes
• Hydrophobity
• Molecular weight
• Size and shape
• Additives
• Bio surfactants

# Wastewater Treatment

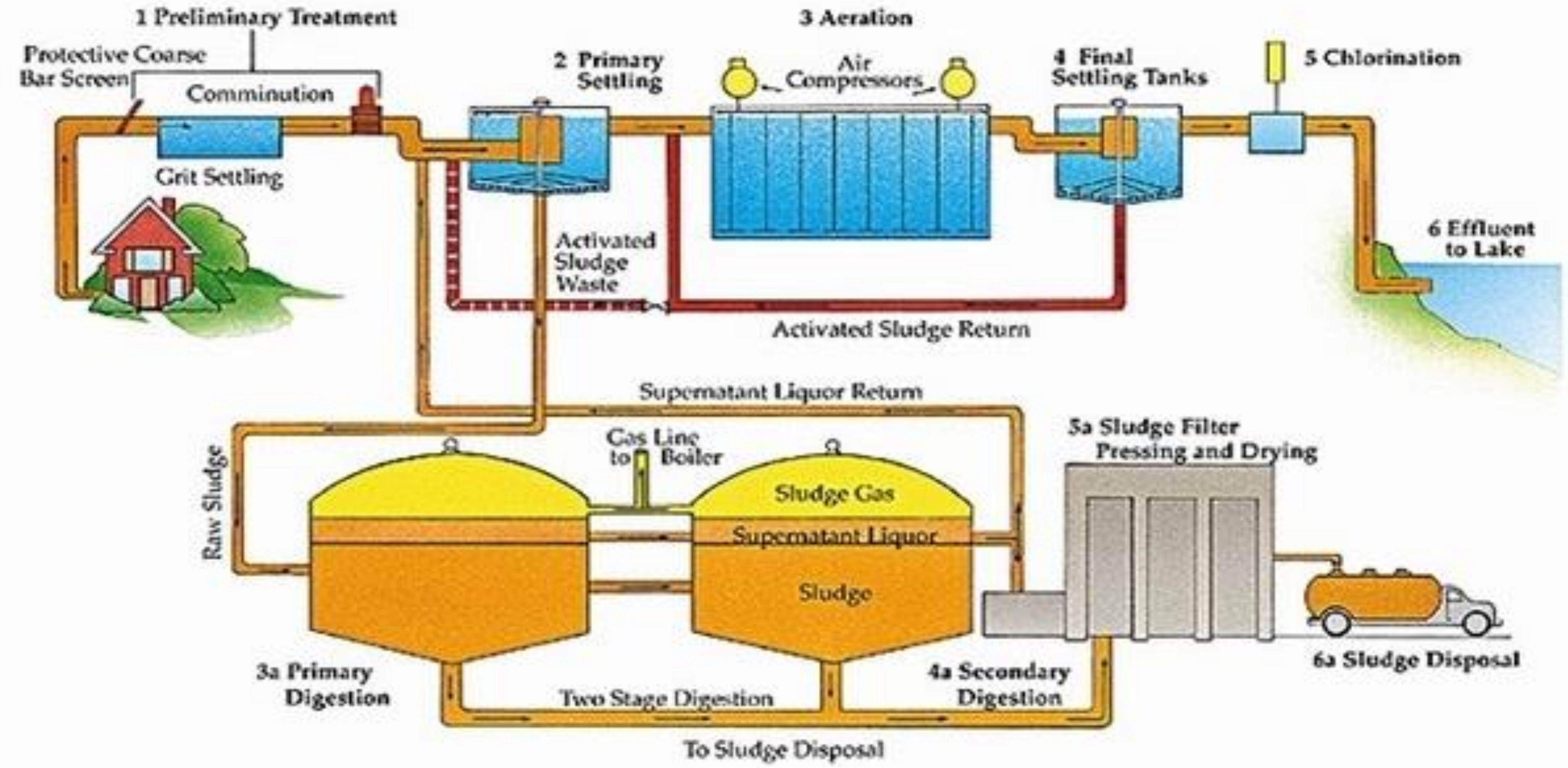
- Water – important for everything – freshwater only 2.5% - Now there is a scarcity of water resources around the world.
- Volume of water being contaminated – many ways
- Waste water originates from – sewage, industrial effluents, agricultural runoff, urban runoff etc.
- Biodegradation in wastewater treatment plants is of environmental importance, because we need to know how much the concentration of organic matter is reduced by the treatment.


Wastewater treatment
mechanical
physicochemical
biological
– drawl
– filtration
– sedimentation
– separation
– flotation
– crystallization
– centrifugation
– sorption
– electrocoagulation
– coagulation and flocculation
– use of membranes
– ozonation
Use of activated sludge:
– oxygen conditions
– anaerobic conditions

# Wastewater Treatment

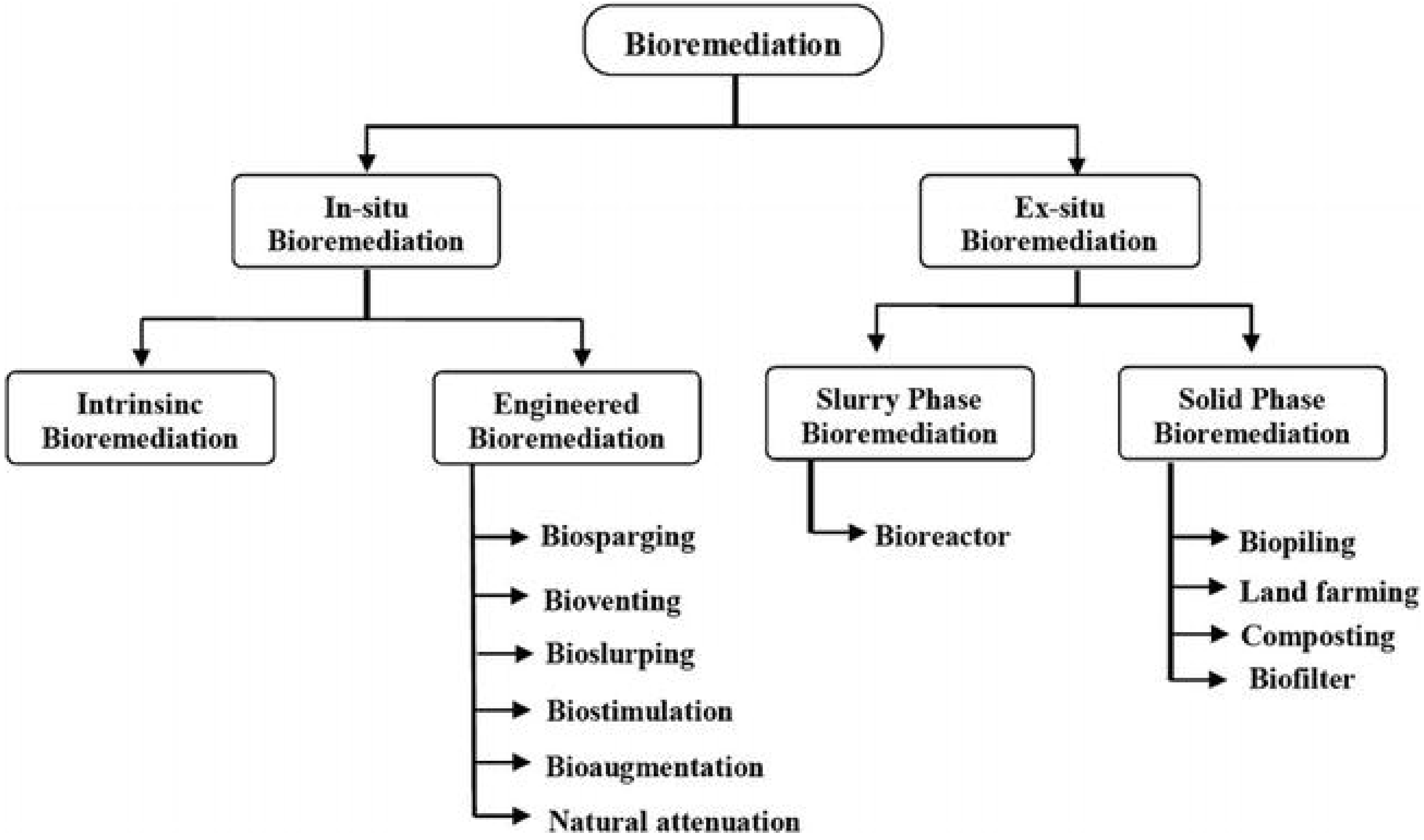


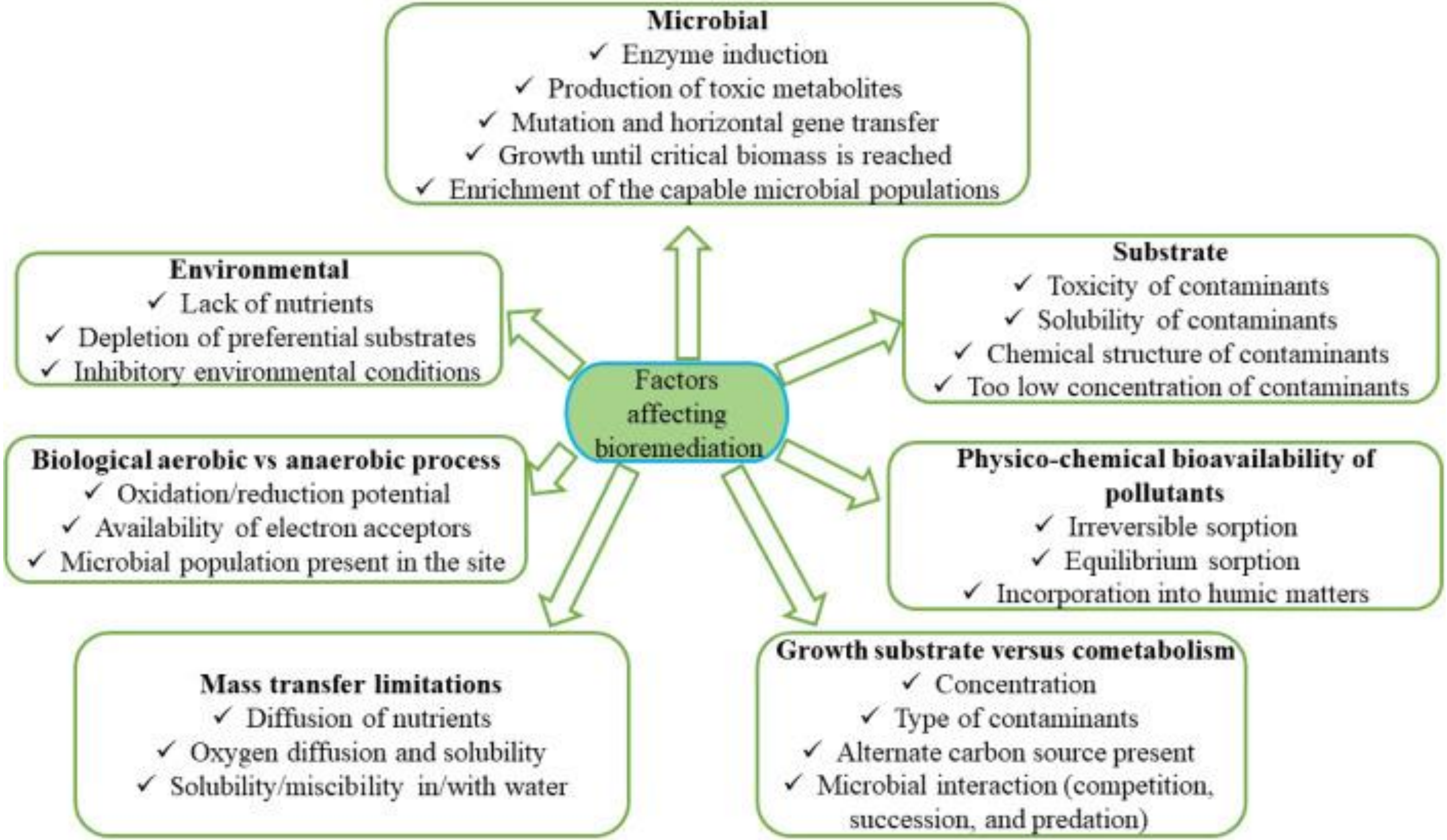
# SEWAGE TREATMENT PROCESS



# 3. Bioremediation

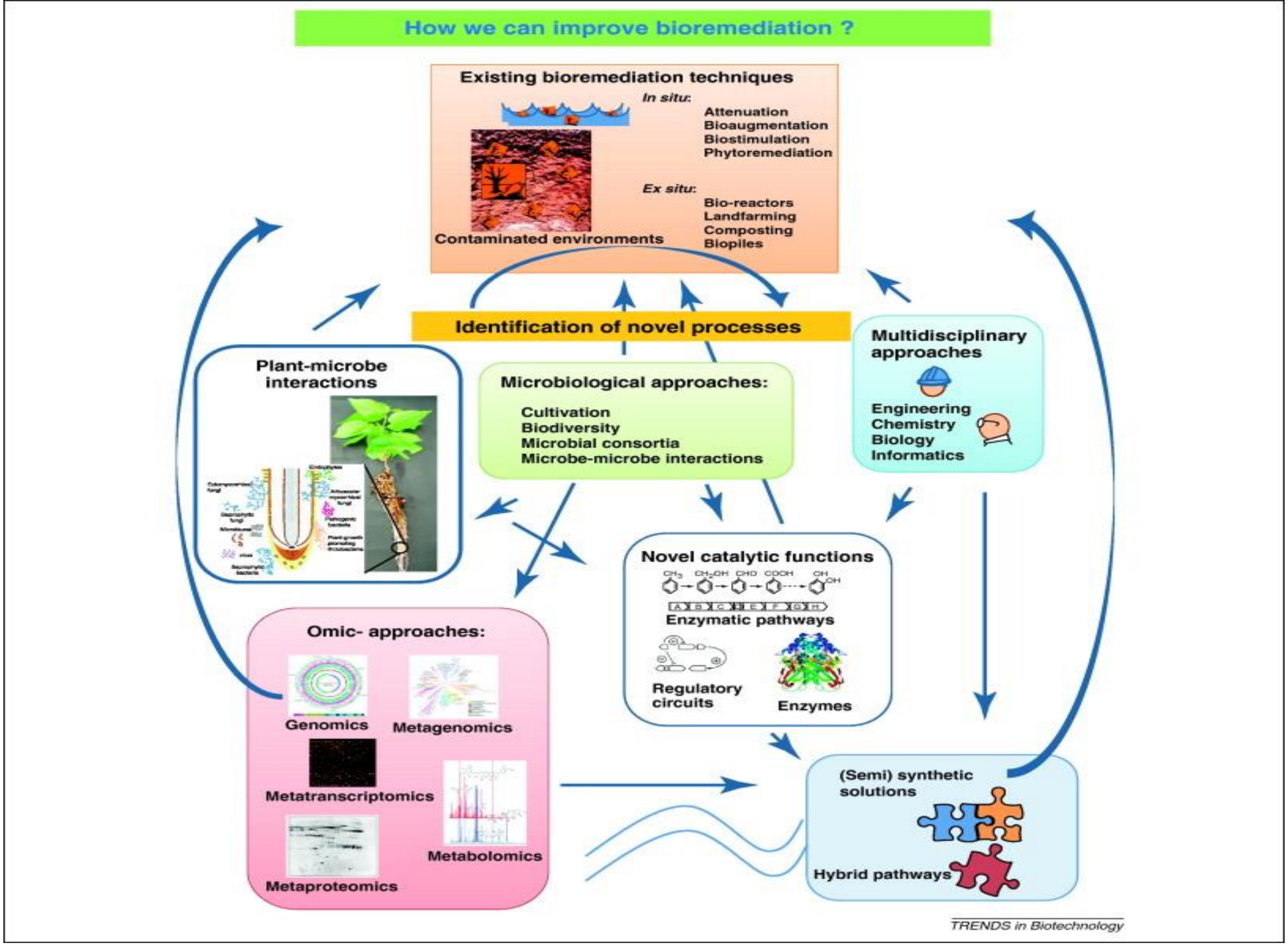
- **Bioremediation** is a **process** where biological organisms are used to remove or neutralize an environmental pollutant by metabolic **process**. The "biological" organisms include microscopic organisms, such as fungi, algae and bacteria, and the "remediation"—treating the situation.
- Microbial **bioremediation** uses microorganisms to break down contaminants by using them as a food source.
- Technologies can generally be classified as in situ or ex situ.
  - *In situ* bioremediation involves treating the contaminated material at the site, e.g. Bio-stimulation and Bioventing
  - *Ex situ* bioremediation involves the removal of the contaminated material to be treated elsewhere, e.g. composting, landfarming and bio-piling
- Types of bioremediation:
  - Phytoremediation,
  - Microbial remediation and
  - Myco-remediation

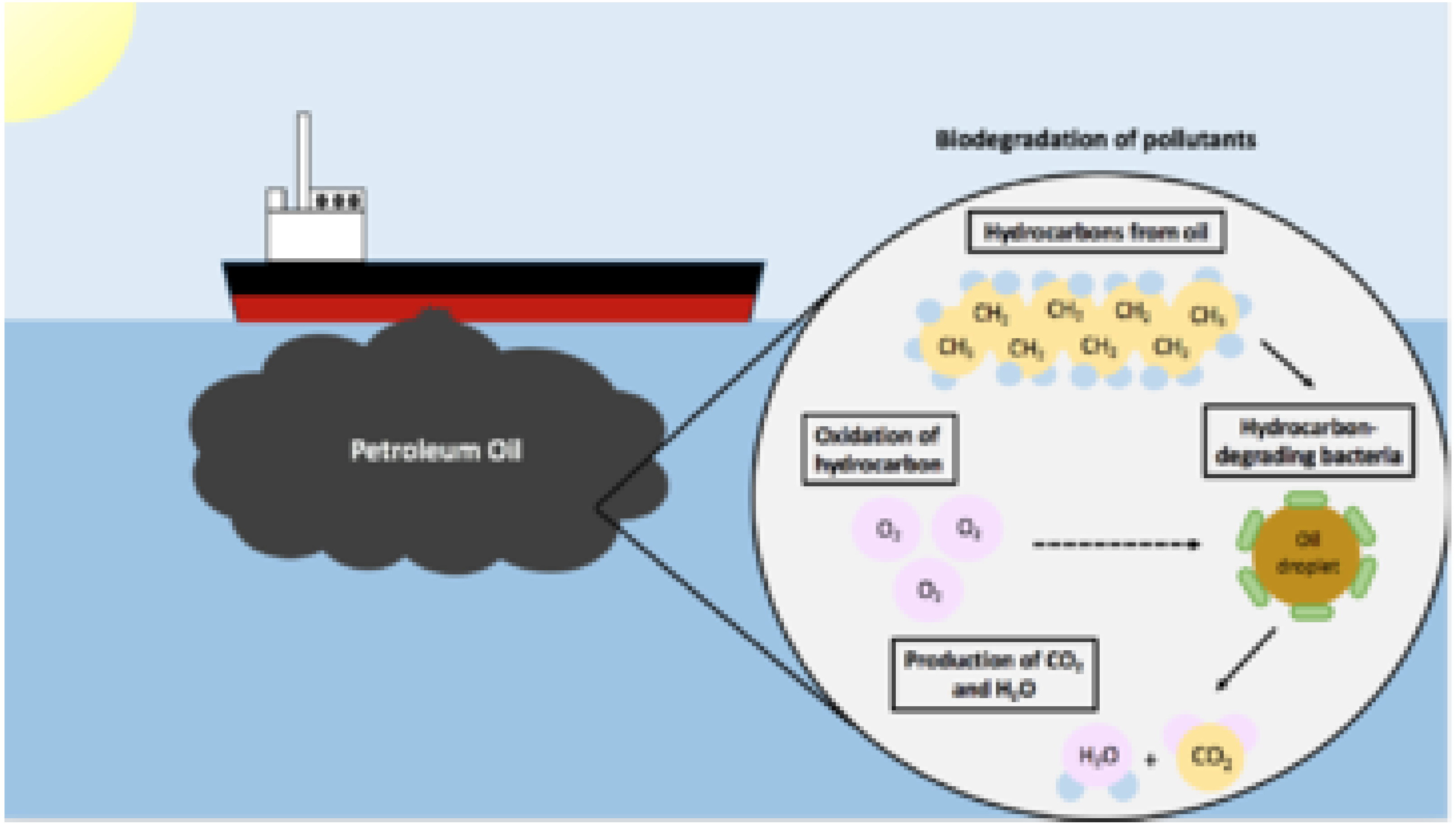

Bioremediation
In-situ Bioremediation
Ex-situ Bioremediation
Intrinsinc Bioremediation
Engineered Bioremediation
Slurry Phase Bioremediation
Solid Phase Bioremediation
Biosparging
Bioventing
Bioslurping
Biostimulation
Bioaugmentation
Natural attenuation
Bioreactor
Biopiling
Land farming
Composting
Biofilter


Microbial
✓ Enzyme induction
✓ Production of toxic metabolites
✓ Mutation and horizontal gene transfer
✓ Growth until critical biomass is reached
✓ Enrichment of the capable microbial populations
Environmental
✓ Lack of nutrients
✓ Depletion of preferential substrates
✓ Inhibitory environmental conditions
Substrate
✓ Toxicity of contaminants
✓ Solubility of contaminants
✓ Chemical structure of contaminants
✓ Too low concentration of contaminants
Factors affecting bioremediation
Biological aerobic vs anaerobic process
✓ Oxidation/reduction potential
✓ Availability of electron acceptors
✓ Microbial population present in the site
Physico-chemical bioavailability of pollutants
✓ Irreversible sorption
✓ Equilibrium sorption
✓ Incorporation into humic matters
Mass transfer limitations
✓ Diffusion of nutrients
✓ Oxygen diffusion and solubility
✓ Solubility/miscibility in/with water
Growth substrate versus cometabolism
✓ Concentration
✓ Type of contaminants
✓ Alternate carbon source present
✓ Microbial interaction (competition, succession, and predation)

# BIODEGRADATION
VERSUS
# BIOREMEDIATION

| BIODEGRADATION | BIOREMEDIATION |
|---|---|
| The breakdown of organic matter by microorganisms, such as bacteria and fungi | The use of either naturally occurring or deliberately introduced microorganisms to consume and break down environmental pollutants, in order to clean a polluted site |
| A natural process | An engineered process |
| Slow process | Fast process |
| Important for the decomposition of materials | Important to clean out environmental pollutants mainly made by man |

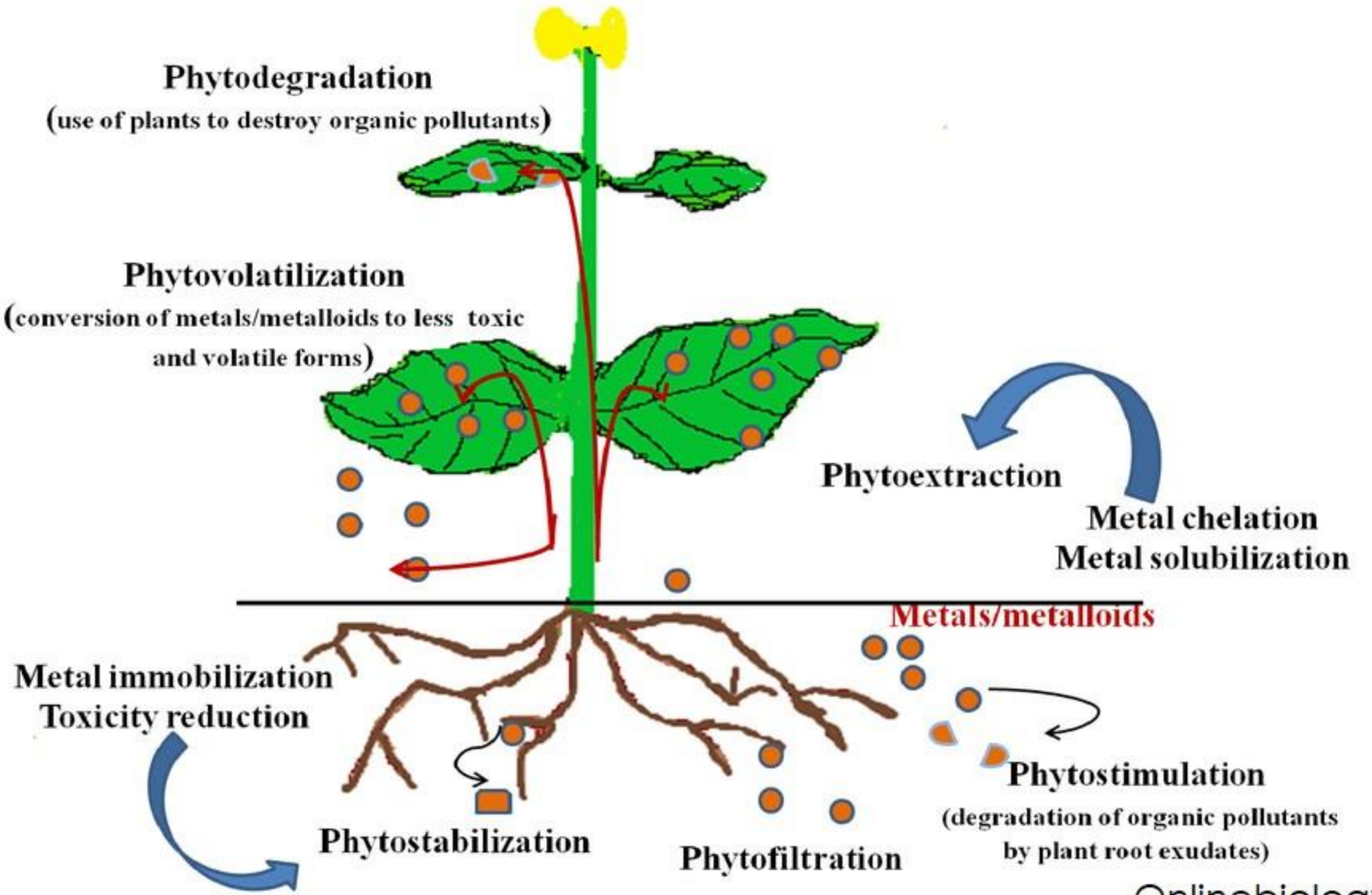

How we can improve bioremediation ?
Existing bioremediation techniques
In situ:
Attenuation
Bioaugmentation
Biostimulation
Phytoremediation
Ex situ:
Bio-reactors
Landfarming
Composting
Biopiles
Contaminated environments
Identification of novel processes
Plant-microbe interactions
Microbiological approaches:
Cultivation
Biodiversity
Microbial consortia
Microbe-microbe interactions
Multidisciplinary approaches
Engineering
Chemistry
Biology
Informatics
Novel catalytic functions
Enzymatic pathways
Regulatory circuits
Enzymes
Omic- approaches:
Genomics
Metagenomics
Metatranscriptomics
Metabolomics
Metaproteomics
(Semi) synthetic solutions
Hybrid pathways
TRENDS in Biotechnology


Petroleum Oil
Biodegradation of pollutants
Hydrocarbons from oil
Hydrocarbon-degrading bacteria
Oil droplet
Oxidation of hydrocarbon
Production of $CO_2$ and $H_2O$
$H_2O$ + $CO_2$

# Phytoremediation

- It involves the interaction of plant roots and the microorganism associated with these root systems to remediate soil containing elevated concentration of organic compounds.
- Alternative to engineering procedures that are usually more destructive to the soil.
- Types of phytoremediation:
  - **Phytoextraction:** based on the ability of certain plants to gradually accumulate contaminants (mainly metals) into their biomass.
  - **Rhizo-filtration:** involve the pumping of contaminated groundwater into troughs filled with the large root systems of appropriate plant species.
  - **Phyto-stabilzation:** immobilize contaminants through adsorption, accumulation, precipitation within the root zone.
  - **Phytodegradation:** attenuation of organic contaminants into less toxic substances within the rhizosphere through biodegradation of soil microbes.
  - **Phytovolatilization:** contaminants taken up by the roots through the plants to the leaves and are volatilized through stomata.

# Phytoremediation

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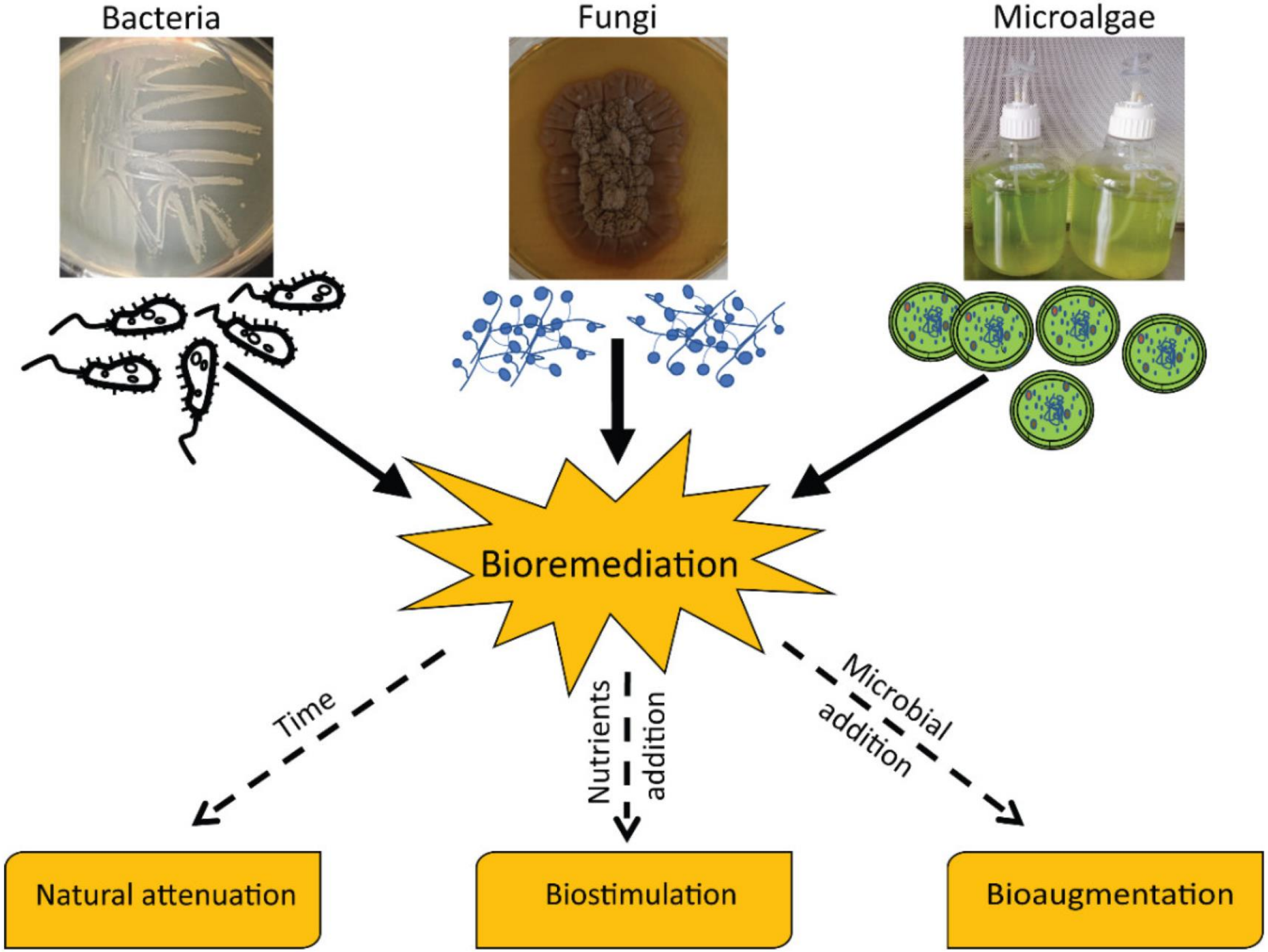
# Microbial Remediation

- Use of microorganisms to degrade organic contaminants and to bind the use of metals in less bioavailable form.

# Mycoremediation

- White rot fungi degrades a wide range of organic molecules that are broadly similar to lignin.
- The release of extracellular lignin-modifying enzymes, with a low substrate specificity so they can act upon various molecules.


Bacteria
Fungi
Microalgae
Bioremediation
Time
Nutrients addition
Microbial addition
Natural attenuation
Biostimulation
Bioaugmentation

**Bioremediation process can be divided into three phases or levels.**

- First, through **natural attenuation**, contaminants are reduced by native microorganisms without any human augmentation.
- Second, **bio-stimulation** is employed where nutrients and oxygen are applied to the systems to improve their effectiveness and to accelerate biodegradation.
- Finally, during **bioaugmentation,** microorganisms are added to the systems. These supplemental organisms should be more efficient than native flora to degrade the target contaminant.

- ❑ **A feasible remedial technology requires microorganisms being capable of quick adaptation and efficient uses** of pollutants of interest in a particular case in a reasonable period of time.
- ❑ Many **factors influence** microorganisms to use pollutants as substrates or co-metabolize them, like, the **genetic potential and certain environmental factors such as temperature, pH, and available nitrogen and phosphorus sources, then, seem to dete**rmine the rate and the extent of degradation.
- ❑ Therefore, applications of (genetically engineered **microorganisms GEM)** in bioremediation have received a great deal of attention. These GEM have higher degradative capacity and have been demonstrated successfully for the degradation of various pollutants under defined conditions. However, ecological and environmental concerns and regulatory constraints are major obstacles for testing GEM in the field.

## A. Natural attenuation

- Natural attenuation or bio-attenuation is the **reduction of contaminant concentrations** in the environment through biological processes (aerobic and anaerobic biodegradation, plant and animal uptake), physical phenomena (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption), and chemical reactions (ion exchange, complexation, abiotic transformation).
- Although, one of the most important components of natural attenuation is biodegradation, the change in form of compounds carried out by living creatures such as microorganisms. Under the right conditions, microorganisms can cause or assist chemical reactions that **change the form of the contaminants so that little or no health risk remains.**
- Natural attenuation occurs at most polluted sites. However, the **right conditions must exist underground to clean sites** properly. If not, cleanup will not be quick enough or complete enough. Scientists monitor these conditions to make sure natural attenuation is working. This is called **monitored natural attenuation or (MNA).** So, Monitored natural attenuation is a technique used to **monitor or test the progress of natural attenuation** processes that can degrade contaminants in soil and groundwater.
- It may be used with other remediation processes as a finishing option or as the only remediation process if the rate of contaminant degradation is fast enough to protect human health and the environment. Natural processes can then mitigate the remaining amount of pollution; regular monitoring of the soil and groundwater can verify those reductions.

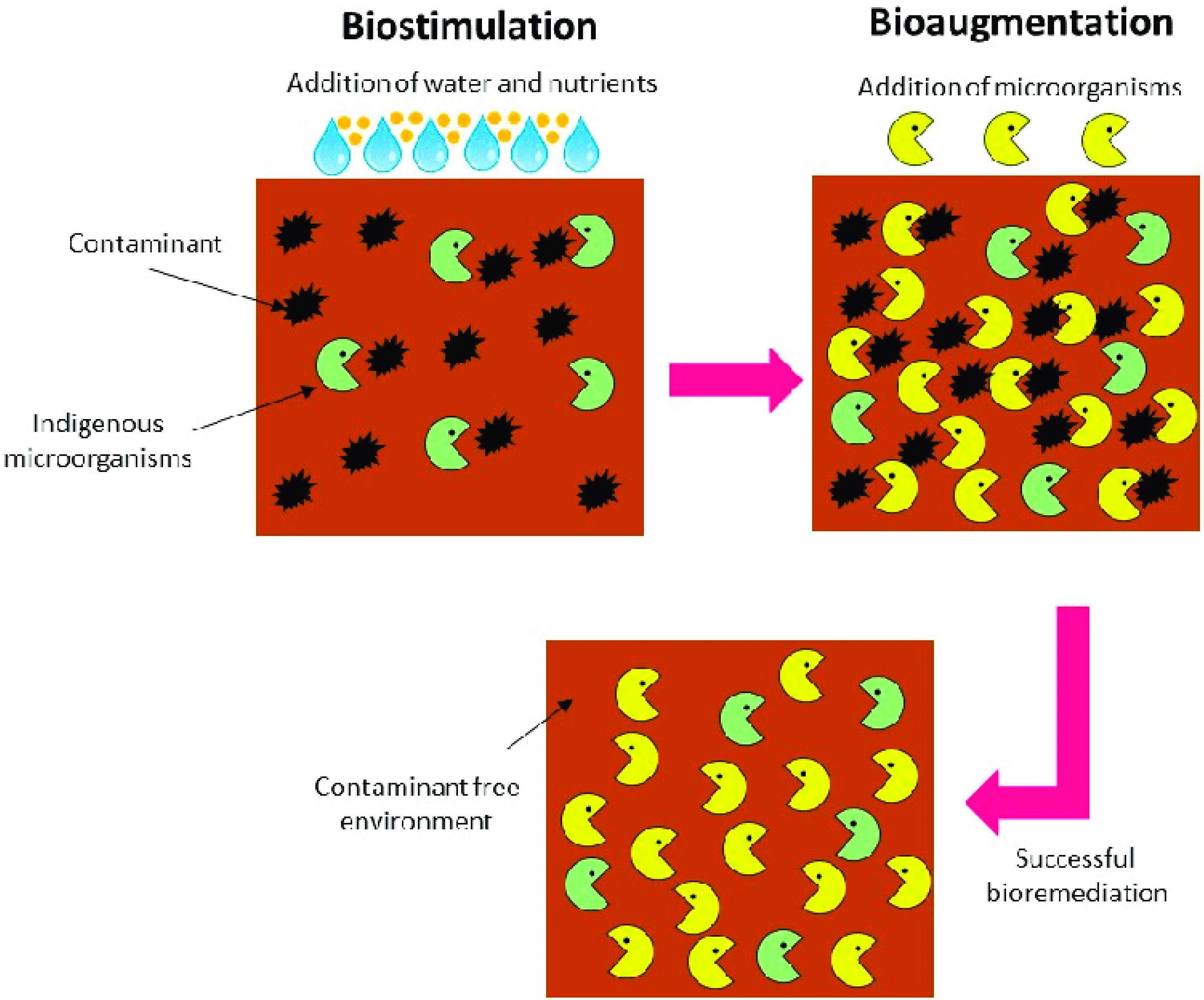
## B. Biostimulation

Bio-stimulation involving the **addition of soil nutrients, trace minerals, electron acceptors, or electron donors enhances the biotransformation of a wide range of soil contaminants**. There are many examples of biostimulation of pollutants biodegradation by **indigenous microorganisms:**

- Trichloroethene and perchloroethene are reported to be completely converted to ethane by microorganisms in a short span of time with the addition of lactate during bio-stimulation.
- Electron shuttles, such as humic substances (HS), may play a significant stimulation role in the anaerobic biotransformation of organic pollutants through enhancing the electron transfer speed. Anthraquinone-2,6-disulfonate (AQDS) from the category of HS can serve as an electron shuttle to promote the reduction of iron oxides and transformation of chlorinated organic contaminants
- Liliane et al. observed that bio-stimulation was more efficient when compared to natural attenuation of biodiesel in contaminated soils. However, the comparative study of Bento et al. revealed that bioaugmentation showed the greatest degradation potential and natural attenuation was more effective than bio-stimulation of soils contaminated with diesel oil.
- Results obtained by Yu et al. indicate that autochthonous microbes may interact and even compete with the enriched consortium during polycyclic aromatic hydrocarbons biodegradation and the natural attenuation appeared to be the most appropriate way to remedy fluorene and phenanthrene contaminated mangrove sediments while bio-stimulation was more capable to degrade pyrene contaminated sediments.

## C. Bioaugmentation

- It is the technique for improvement of the capacity of a contaminated matrix (soil or other biotope) to remove pollution by the **introduction of specific competent strains or consortia of microorganisms**.
- The basic premise for this intervention is that the metabolic capacities of the **indigenous microbial community already present in the biotope slated for cleanup will be increased by an exogenously enhanced genetic diversity, thus leading to a wider repertoire of productive biodegradation reactions.**
- Moreover, genetically engineered microorganisms (**GEMs) exhibiting enhanced degradative capabilities** encompassing a wide range of aromatic hydrocarbons have also potential for soil bioaugmentation.
- Many studies have shown that both **abiotic** and biotic factors influence the effectiveness of bioaugmentation, the most important **abiotic factors are temperature, moisture, pH and organic matter content, however, aeration, nutrient content and soil type also determine the efficiency of bioaugmentation**. **Biotic factors, including competition between indigenous and exogenous microorganisms for limited carbon sources as well as antagonistic interactions and predation by protozoa and bacteriophages, also play essential roles in the final results of bioaugmentation.**
- Bioaugmentation-assisted phytoextraction using PGPR or AMF is also a promising method for the cleaning-up of soils contaminated by metals


Biostimulation
Addition of water and nutrients
Bioaugmentation
Addition of microorganisms
Contaminant
Indigenous microorganisms
Contaminant free environment
Successful bioremediation

# Bioaugmentation vs Biostimulation

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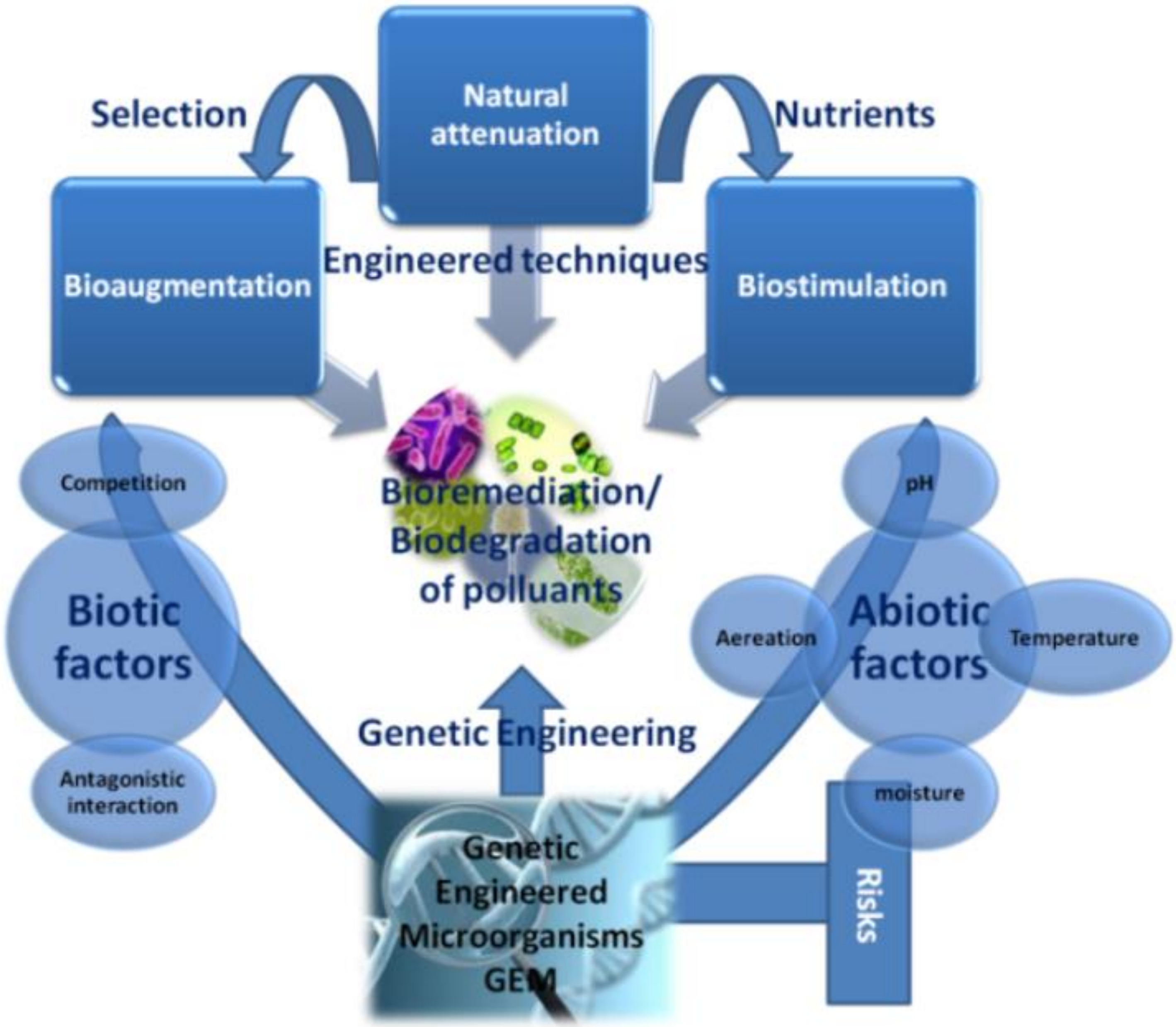
| | Bioaugmentation | Biostimulation |
|---|---|---|
| DEFINITION | Bioaugmentation is the process of adding specific microorganisms to enhance the existing populations and promote the biodegradation process | Biostimulation is the process of adding electron acceptors, electron donors, or nutrients to stimulate naturally occurring microbial populations in the contaminated area |
| UTILIZING MICROORGANISMS | Exogenous microorganisms | Indigenous microorganisms |
| ADDITION | Cultured microorganisms | Nutrients and electron acceptors mainly |
| DRAWBACKS | The introduced microbe often cannot be established in the environment and these introduced organisms rarely survive in the new environment | Due to contaminant toxicity, the existing microbial population may not be enough for the biodegradation process |




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