MICROBIAL BIOTECHNOLOGY

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BIOPOLYMERS

BIOPESTICIDES

BIOSURFACTANTS

BIODEGRADATION

BIOPOLYMERS

- Polymers are a class of “giant” molecules consisting of discrete building blocks linked together to form long chains.
- Some natural polymers, such as nucleic acids and proteins, carry and manipulate essential biological information, while other polymers such as the polysaccharides—provide fuel for cell activity and serve as structural elements in living systems.
- Biopolymers are large, multiunit macromolecules synthesized by microorganisms, plants, and animals.
- Some of these polymers have potential applications in virtually all sectors of the economy.
- For example, they can be used as adhesives, absorbents, lubricants, soil conditioners, cosmetics, drug delivery vehicles, textiles, high-strength structural materials, and even computational switching devices.

Table 1: Biopolymers found in nature and their functions

Polymer	Monomers	Function(s)
Nucleic acids (DNA and RNA)	Nucleotides	Carriers of genetic information universally recognized in all organisms
Proteins	Alpha-ammo acids	Biological catalysts (enzymes), growth factors, receptors, structural materials (wool, leather, silk, hair, connective tissue); hormones (insulin); toxins; antibodies
Polysaccharides (carbohydrates)	Sugars	Structural materials in plants and some higher organisms (cellulose, chitin); energy storage materials (starch, glycogen); molecular recognition (blood types), bacterial secretions
Polyhydroxyalkanoates	Fatty acids	Microbial energy reserve materials
Polyphenols	Phenols	Structural materials in plants (Iignin), soil structure (humics, peat), plant defense mechanisms (tannins)
Polyphosphates	Phosphates	Inorganic energy storage materials
Polysulfates	Sulfates	Inorganic energy storage materials

Some polymers produced by microbial systems

1. XANTHAN GUM

This is a high-molecular-weight exopolysaccharide produce by *Xanthomonas campestris;* a gram-negative obligatory aerobic soil bacterium that produces xanthan gum (biopl oymer), as a by-product of its metabolism.

(a) It has high viscosity

(b) It is stable in extreme physical and chemical environments

(c) It exhibits physical and chemical properties similar to those of a plastic.

In particular, its physical properties make it useful as a:

(a) stabilizing,

(b) emulsifying,

(c) thickening,

(d) suspending agent.

2. Melanin

- ❖ Melanins are a large, diverse family of light-absorbing biopolymers that are synthesized by animals, plants, bacteria, and fungi.

- ❖ They are irregular, somewhat random polymers that are composed of indoles, benzthiazoles, and amino acids

- ❖ It has been suggested that these pigments might be useful as:

(a) Topical sunscreens,
(b) Sunlight-protective coatings for plastics,
(c) Additives for cosmetic products.

- ❖ The first step in their synthesis is the oxidation of tyrosine to dihydroxyphenylalanine quinone, which is catalyzed monooxygenase tyrosinase,

- ❖ The final stages of the polymerization of melanin are non enzymatic, and depending on the chemical nature of the non quinone components that are incorporated into the polymeric structure (typically hydroxylated organic compounds),

- ❖ The end product can be black, brown, yellow, red, or violet.

3. Adhesive Protein

- The blue mussel *Mytilus edulis originally produces* an adhesive protein biopolymer, This biopolymer is an exceptionally strong, waterproof adhesive protein, called byssal adhesive, that enables the mussel to attach very tightly to a variety of surfaces. Following its secretion, the byssal adhesive becomes highly cross-linked (randomly), and consequently, the protein cannot be sequenced.

- However, it was possible to isolate an intracellular precursor form of the adhesive protein, called the 130-kilodalton (kDa) precursor protein, that can be analyzed biochemically.

- It was found that the 130-kDa precursor protein is rich in serine, threonine, lysine, proline (Pro), and tyrosine; 60 to 70% of the amino acids contain a hydroxyl group.

- Most of the proline residues are hydroxylated to either 3- or 4-hydroxyproline (Hyp), and the majority of the tyrosines are hydroxylated to 3,4-dihydroxyphenylalanine (DOPA).

- A higher number of DOPA residues is believed to result in a protein with greater adhesive properties. Unfortunately, the adhesive properties of this protein make it extremely difficult to purify (i.e., it sticks to everything), thereby limiting its commercial possibilities.

4. Rubber

- Natural rubber, *cis*-1, 4-polyisoprene, is an extensively used biopolymer that is obtained from a large number of different plants.

- The biosynthesis of rubber starts from simple sugars and requires approximately 17 enzyme catalysed steps,

- The final step being the polymerization of isopentenyl pyrophosphate onto an allylic pyrophosphate is catalysed by the enzyme rubber polymerase.

- E.g of rubber producing plant *Hevea brasiliensis*

5. Polyhydroxyalkanoates

- Polyhydroxyalkanoates are class of biodegradable polymers that are produced by a number of different microorganisms, most notably *Alcaligenes eutrophus.*
- These compounds have thermoplastic or elastic properties, depending on the polymer composition, and are being considered for use in the synthesis of a range of biodegradable plastics.

- Poly (3-hydroxybutyric acid) is the most thoroughly studied and characterized polyhydroxyalkanoate. Both the polymer and the *A. eutrophus* genes that encode its synthesis have been characterized.

- Poly(3-hydroxybutyric acid), its copolymer [poly(3-hydroxybutyrate-co-3-hydroxyvalerate)], and another polyhydroxyalkanoate [poly(3-hydroxyvaleric acid)] are produced commercially in the United Kingdom by the fermentation of *A. eutrophus*.

BIOPESTICIDES

Bioinsecticides

- Bio-pesticides are those biological agents that are used for control of weeds, insects and pathogens.

- Bio-pesticides are pest management tools that are based on beneficial microorganisms (bacteria, viruses, fungi and protozoa)

- They include naturally occurring substances that control pests (biochemical pesticides)

- Microorganisms that control pests (microbial pesticides)

- Pesticidal substances produced by plants containing added genetic Plant-Incorporated Protectant (PIP) materials

Bio-pesticides are of two types: bio-herbicides and bio-insecticides

Bio-insecticides

- Bio-insecticides are those biological agents that are used to control harmful insects. They include the following.

(a) Predators

Destructive insects or plant pests can be brought under control through introduction of their natural predators.

The predators should be specific and unable to harm the useful insects.

E.g: Introduction of ladybugs (Lady Bird Beetles) and Praying Mantis has been successful in combating scale insects or aphids which feed on plant sap

(b) Natural Insecticides: They are insecticides and related pesticides which are obtained from microbes and plants.

A number of natural insecticides are available

The common ones include:

(i) **Azadirachtin:** from Margosa or Neem (*Azadirachta indica*). It occurs in Margosa extract. Spray of the same keeps away the Japanese beetles and other leaf eating pests because of the antifeedant property of azadirachtin.

(ii) **Rotenones**: They are powerful insecticides which are harmless to warm blooded animals. Chinese are believed to be first to discover their insecticidal properties. Rotenones are obtained from the roots of *Derris elliptica* and *Lonchocarpus nicou*

(iii) **Squill:** The red variety of Sea Onion (Red Squill, *Ureginea maritima*) produces a radicide which does not have any harmful effect on other animals

(iv) **Nicotine**: It is obtained from Nicotiana species. The purified chemical is highly poisonous. Nicotine sulphate is one of the most toxic insecticides

(v) Pyrethrum: It is an insecticide which is obtained from the inflorescence of *Chrysanthemum cinerarifolium* (*Dalmation pyrethrum*), *C. coccineum* and *C. marshallii*. The active compounds are pyrethrin and cinerin. Pyrethrin is also used in fly sprays, aerosols, mosquito coils, etc.

(vi) Thurioside: It is a toxin produced by bacterium *Bacillus thuringenesis*. The toxin is highly effective against different groups of insects like moths, flies, mosquitoes and beetles. It does not cause any adverse environmental pollution or disturbance.

- Thurioside occurs as crystals in the bacterium. It kills the susceptible insects through inhibiting ion transport in the midgut, formation of pores in gut epithelium, swelling and bursting of cells,

(**vii) Transgenic Plants:** They are crop plants which are modified through genetic engineering to develop natural resistance to insects by inserting cry genes of *Bacillus thuringenesis* into them,

e.g., Bt Cotton. Similarly, transgenic Tomato has been developed which is resistant to homworm larvae.

Characteristics of Biopesticides

1. They have a narrow target range.

2. They have a specific mode of action.

3. They are slow acting and have relatively critical application times.

4. They can suppress, rather than eliminate a pest population.

5. They have limited field persistence and a short shelf life.

6. They are safer to humans and the environment than conventional pesticides.

7. They have a short residual effect

Advantages of Biopesticides

1. Biopesticides are inherently less harmful in comparison to convectional pesticides.

2. Biopesticides are designed to affect only one specific pest or in some cases, a few organisms, in contrast to convectional pesticides, that may affect organisms as different as birds, insects and mammals, across a broad spectrum.

3. They are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by convectional pesticides.

4. When used as a component of Integrated Pest Management(IPM) programmes, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high

Biosurfactants

BIOSURFACTANTS

- Surfactants are surface-active agents that are produced through chemical reactions

- Biosurfactants are surface-active agents that are produced by microorganisms (especially bacteria and yeast) extracellularly or produced on living surfaces mainly on surfaces of microorganisms or as part of their cell membrane

- They are amphiphatic in nature and it contains both hydrophilic and hydrophobic moieties which reduce the surface and interfacial tension of the surface and interface respectively
- hydrophobic moieties are usually based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic moieties can be a phosphate, amino acid, carbohydrate, or cyclic peptide

Properties of Biosurfactants

Biosurfactant have several advantageous properties in contrast with chemical surfactants, these includes:

- ❖ they possess no or lesser toxicity
- ❖ they are highly biodegradable
- ❖ better environmentally compatible and non hazardous,
- ❖ higher foaming properties
- ❖ there activities are highly selective and specific even at extreme temperatures, pH and salinity and
- ❖ also they have the ability to be synthesized from renewable feed-stock

Classification of biosurfactants

- Unlike the chemically produced surfactants that are classified according to the type of the polar group present and their dissociation pattern in water;

biosurfactants are classified by their:

- ❖ Microbial origin,
- ❖ Mode of action,
- ❖ Chemical composition,
- ❖ Molecular weight,
- ❖ Physico-chemical properties.

Taking into account their molecular weight they are partitioned into:

(a) Low molecular mass and (b) high molecular mass biosurfactant

(a) low-molecular mass biosurfactants: include the glycolipids, phospholipids, and lipopeptides, they are efficient in reducing surface and interfacial tensions.

(b) High-molecular mass biosurfactants/bioemulsifiers include the particulate surfactants, amphipathic polysaccharides, lipopolysaccharides, proteins, lipoproteins and other complex mixtures of these biopolymers.

- The biosurfactants with high-molecular-mass are more effective and efficient at stabilizing oil-in-water emulsions (that is, they act as emulsion-stabilizing agents).

- They do these by accumulating at the interface between two immiscible liquids or between a fluid (liquid) and a solid thereby lowering surface (liquid-air) and interfacial (liquid-liquid) tension, by reducing the repulsive forces between two non similar phases thus allowing these two phases to mix and interact more easily

Table 2: Major classes of biosurfactants and microorganisms involved

Surfactant class	Microorganism	
Glycolipids		Low molecular weight biosurfactants
Rhamnolipids	*Pseudomonas aeruginosa*	
Trehalose lipids	*Arthobacter* sp., *Rhodococcus erythropolis*	
Sophorolipids	*Candida bombicola, C. apicola*	
Mannosylerythritol lipids	*C. antartica*	
Lipopeptides		
Lichenysin	*Bacillus licheniformis*	
Viscosin	*P. fluorescens*	
Surfactin/iturin/fengycin	*B. subtilis*	
Serrawettin	*Serratia marcescens*	
Surface-active antibiotics		
Gramicidin	*Brevibacterium brevis*	
Polymixin	*B. polymyxa*	
Antibiotic TA	*Myxococcus Xanthus*	

Surfactant class	Microorganism	
Fatty acids or neutral lipids		
corynomicolic acids	*Corynebacterium insidibasseosum*	
Polymeric surfactants		
Emulsan	*Acinetobacter calcoaceticus*	**High molecular weight biosurfactants**
Alasan	*A. radioresistens*	
Liposan	*C. lipolytica*	
Lipomanan	*C. tropicalis*	
Particulate biosurfactants	*A. calcoaceticus*	
	Cyanobacteria	

Applications of Biosurfactants

1. Biosurfactants are ecologically safe and can be applied in wastewater treatment
2. Bioremediation of soil
3. Removal of heavy metal from contaminated soil
4. Microbial enhanced oil recovery (MEOR)
5. Hydrocarbon degradation in the soil environment, in aquatic environment and hexa-chloro cyclohexane degradation

6. Potential food applications

- ❖ Food-formulation ingredients
- ❖ Anti adhesive agents

7. Applications of biosurfactants in pharmaceutics and medicine

- Therapeutic and biomedical applications and antimicrobial activity
- Anti adhesive agents
- Immunological adjuvants

8. Anticancer activity

Role of Biosurfactants in Biodegradation Processes

- ❖ A better technique that can effectively enhance the remediation of hydrocarbon contaminated environments is the use of biosurfactants.

- ❖ They influence hydrocarbon degradation in two ways:
- ❖ The first includes; increase the substrate availability for microbial utilization,

- ❖ while the second process involves cell surface interaction which elevates the hydrophobicity of the surface permitting the hydrophobic substrates to associate more easily with bacterial cells.

- ❖ These reduction of surface and interfacial tensions by biosurfactants results in the increase of surface areas of insoluble compounds leading to increased bioavailability and mobility of hydrocarbons.

Mechanisms of hydrocarbon removal by biosurfactants

- Addition of biosurfactants can be expected to enhance hydrocarbon biodegradation by mobilization, solubilisation or emulsification (Figure 1)

- Mobilization mechanism of biosurfactant in hydrocarbon removal usually takes place at concentrations below the biosurfactant CMC, at such concentrations; biosurfactants reduce the surface and interfacial tension between air/water and soil/water systems. Due to the reduction of the interfacial force, contact of biosurfactants with soil/oil system increases the contact angle and reduces the capillary force holding oil and soil together.

- In turn, above the biosurfactant CMC the solubilisation (that is, incorporation of these molecules into a micelle) process takes place. At these concentrations biosurfactant molecules associate to form micelles, which dramatically increase the solubility of oil, the hydrophobic ends of the biosurfactant molecules then connect together inside the micelle creating an environment compatible for hydrophobic organic molecules while the hydrophilic ends becomes exposed to the aqueous phase on the exterior

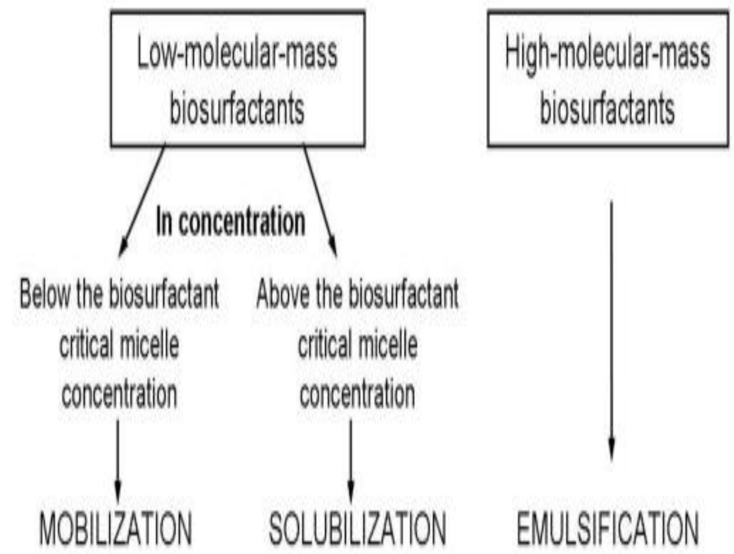

Figure 1: Mechanisms of hydrocarbon removal by biosurfactants depending on their molecular mass and concentration

NOTE

- ❖ The efficiency of surfactants is commonly measured using the CMC and an efficient biosurfactants usually have low CMC, this means that, little quantity of biosurfactant is required to decrease the surface tension.

- ❖ Formation of Micelle has a very significant role in microemulsion formation (these microemulsions are stable and clear liquid mixtures of oil domains and water separated by monolayer or aggregates of biosurfactants

BIODEGRADATION

DEGRADATION: This is the breaking down of large molecules, compound, or materials into smaller particle.

- When living organisms are involved it is called biodegradation, therefore
- Biodegradation can be defined as the breakdown of complex compound into smaller ones or transformation of one compound to another by living organisms (plant, microrganisms like bacteria, fungi)
- The process of biodegradation occurs naturally and it is usually a very slow process.
- When modern technology is used to quicken or modify the process of naturally slow biodegradation, the process is called bioremediation.
- Bioremediation therefore refers to the productive and efficient use of microorganisms to remove or detoxify pollutants usually as contaminants of soil, water or sediment that otherwise threatens public health.
- Bioremediation therefore cannot achieve any process that is impossible to achieve with natural biodegradation.

- **Bio: life; Remediate: to solve a problem**

- **Bio-remediate: the use of living things to solve problem**

- Therefore bioremediation simply means the use of living organisms (usually bacteria, fungi, actinomycetes, cyanobacteria and to a lesser extent-plants) to reduce, degrade, eliminate or detoxify, toxic pollutants or contaminate from an environment

Strategies of Bioremediation

There are two general bioremediation strategies

BIOSTIMULATION

This is the introduction of additional nutrients or products into a contaminated site, which increases the population of the indigenous microorganisms.

For example, petroleum-degrading bacteria are naturally present in seawater, but they degrade oil at a very slow rate because the low levels of nitrogen and phosphorus in the environment limit their growth. To enhance bioremediation of oil spills. A fertilizer containing these nutrients, and which adheres to oil, was developed. When this fertilizer is applied to an oil spill, microbial growth is stimulated, leading to at least a threefold increase in the speed of degradation by bacteria.

BIOAUGMENTATION

- This involves the addition of specifically formulated microorganisms to a contaminated site. It is done in conjunction with the development and monitoring of an ideal growth environment, in which these selected bacteria can live and work.

- The basic premise for this intervention is that the metabolic capacities of the indigenous microbial community already present in the contaminated site will be increased by an exogenously enhanced genetic diversity, thus leading to a wider repertoire of productive biodegradation reactions

Two factors limit the use of added microbial cultures in a land treatment unit:

1. nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and

2. most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

Types of bioremediation

There are two different types of bioremediation,

In situ bioremediation

- This involves the treatment of the contamination on site.

- In the case of soil contamination, in situ bioremediation involves the addition of mineral nutrients. These nutrients increase the degradation ability of the microorganisms that are already present in the soil.

- Sometimes new microorganisms are added to the contaminated area. Microorganisms can sometimes be genetically engineered to degrade specific contaminants.

Ex Situ Bioremediation

- *Ex situ* techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water)

- Ex situ bioremediation involves the physical extraction of the contaminated media to another location for treatment.

- If the contaminants are just in the soil, the contaminated soil is excavated and transported for treatment.

- If the contamination has reached the groundwater, it must be pumped and any contaminated soil must also be removed.

- One major thing that this removal of the contaminants does right away is stop the spread of the contamination

FACTORS AFFECTING BIOREMEDIATION

Nutrients availability

- Although the microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. This is done by adding nutrient to help indigenous microorganisms.
- These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants.
- All of them will need nitrogen, phosphorous, and carbon

- Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells

Environmental requirements

- Optimum environmental conditions for the degradation of contaminants are needed.
- Microbial growth and activity are readily affected by pH, temperature, and moisture.

pH

- ❖ Nearly all groundwater falls in the pH range 6 to 9 ,but soil pHs can be acidic in areas where sufficient rainfall occurs to leach bases from the soil or alkaline, primarily in arid and semiarid regions. Soil and groundwater with low alkalinity may also become acidic due to contaminant biodegradation (e.g., through production of organic acids or HCl from reductive dehalogenation).

- ❖ Fungi prefer acidic conditions , whereas bacterial biodegradation rates tend to be fastest at nearly neutral pHs.

- ❖ Therefore, lime is commonly added to neutralize the pH where acidity is of concern (i.e. If the soil has too much acid it is possible to rinse the pH by adding lime).

- ❖ Microbial oxidation of reduced sulphur compounds generates protons and can be used to lower the pH of alkaline environments

Temperature

Temperature affects biochemical reactions rates, and the rates of many of them double for each 10 °C rise in temperature. Above a certain temperature, however, the cells die.

Plastic covering can be used to enhance solar warming in late spring, summer, and autumn.

- ❖ In general, biodegradation rates decrease with temperature; however, temperature effects can be complex.
- ❖ For example, temperature influences hydrocarbon biodegradation by causing changes in:

1. the physical nature and chemical composition of the hydrocarbons,
2. the rate of hydrocarbon metabolism by microorganisms, and
3. the microbial community composition

Moisture

Inadequate moisture levels can severely restrict biodegradation in surface soils, which are subject to

- ❖ The optimum moisture level for a given situation is a function of the soil properties, contaminant characteristics, and oxygen requirements.

- ❖ When moisture levels are high, there is less air-filled pore space and the soil soon becomes anaerobic, due to the slow rate of oxygen diffusion through water. However, degradation rates also decrease if the moisture levels become too low.

- ❖ Available water is essential for all the living organisms, and irrigation is needed to achieve the optimal moisture level, or sprinkling.

Oxygen

- The amount of available oxygen will determine whether the system is aerobic or anaerobic. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorinated compounds are degraded only in anaerobic ones. To increase the oxygen amount in the soil it is possible to till or sparge air. In some cases, hydrogen peroxide or magnesium peroxide can be introduced in the environment.

- Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as gypsum or organic matter can be applied.
- Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soils with low permeability may not be appropriate for *in situ* clean-up techniques.

Contaminant Availability

Bioremediation strategies designed to increase microbial activity through the addition of key substrates or modification of environmental conditions will not have a positive impact if the microorganisms cannot access the contaminant (i.e., it is not bioavailable).

ADVANTAGES OF BIOREMEDIATION TECHNOLOGY

a. Bioremediation technology is a natural process with no harmful end products. when the contaminant is degraded, the population of these microbes declines drastically. The residues left after bioremediation are usually harmless products such as carbon dioxide, water and biomass.

b. Bioremediation is considered to be useful for complete destruction of a wide variety of contaminants. Many hazardous compounds can be transformed to harmless products. This eliminates the chances of future liability associated with treatment and disposal of contaminated material

c. Instead of transferring contaminants from one environmental medium to another, bioremediation accomplishes complete destruction of the target pollutants at the pollution site.

d. Bioremediation can often be carried out on site without causing a major disruption of normal site activities.

e. Bioremediation can be less expensive when compared with other remedial technologies used for cleaning up hazardous wastes.

Disadvantages of Bioremediation technology

1. Bioremediation technology is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete microbial degradation.

2. Bioremediation process is usually very slow and time consuming, they take longer time than other treatment methods such as excavation or removal of soil by incineration.

3. Bioremediation processes are highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions and appropriate levels of nutrients and contaminants.