Biosurfactant: Types, Detection Methods, Importance and Applications

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Abstract

There are many insoluble substrates that remain unavailable for macro and micro organisms. A microorganism synthesizes surface active agents to utilize these substrates that are collectively called as biosurfactants. Biosurfactants enhances the surface area of hydrophobic water-insoluble substrates that organism utilizes it for its growth and work efficiently in process of biodegradation, bioremediation and biocontrol. Oil spilling is a distressing problem for the environment that is affecting chemical, physical and biological equilibrium of all systems. A microorganism does many miraculous jobs. In such oil spilling conditions, they are personalized to grow in environmental conditions that are rich in oil. Their ability of utilizing oil we cannot ignore that highlights its importance to utilize the hydrocarbons as sole sources of energy. The hydrocarbon-utilizing microorganisms generates surface active agent that accelerates the process that are best defined as biosurfactants. Many methods are there to check the biosurfactants potentiality of the bacterial isolates such as Hemolytic activity, Drop collapsing test, Oil spreading test, Emulsification index test, Blue agar plate or CTAB agar plate method and Hydrocarbon overlay agar method. In this paper its classification, properties, methods and significance are presented. The paper also highlights its importance in reducing the oil spills.

Key words: Biosurfactants, Bioremediation, Emulsifiers, Hydrocarbon-degrading microorganisms.

Access this article online	
Quick Response Code:	**Website:** www.innovativepublication.com
	DOI: 10.5958/2394-5478.2016.00002.9

Introduction

Biologically synthesized surface active agents are known as biosurfactants. They are synthesized by small sized organisms known as microorganisms. Biosurfactants are amphiphilic compound that have hydrophilic and hydrophobic domains. The hydrophilic domains are usually consisting of carbohydrates, amino acids and phosphate groups. Hydrophobic domains are usually made up of long chain fatty acids.

Classification of biosurfactants

The biosurfactants are classified on the basis of its molecular weight and on the basis of its chemical composition.

1. Classification based on molecular weight

Low molecular- weight biosurfactants: These compounds lower the surface and interfacial tension at the air/water interfaces (Salihu *et al.*, 2009). The low molecular- weight biosurfactants are generally glycolipids or lipopeptides. The glycolipids are till date best studied as rhamnolipids, trehalolipids and sophorolipids which are disaccharides that are acylated with long- chain fatty acids or hydroxyl fatty acids (Ron and Rosenberg, 2001).

High- molecular weight biosurfactants: These are most commonly referred to as bioemulsan (Salihu *et al.*, 2009). They are more effective in stabilizing oil in water emulsions. They are highly efficient emulsifiers that work at low concentrations. It also bears extensive substrate specificity (Dastgheib *et al.*, 2008). Ron and Rosenberg (2001) reported that a large number of bacterial species from different genera produce exo-cellular polymeric surfactant composed of polysaccha-rides, proteins, lipopolysaccharides, lipo-proteins or complex mixtures of these biopolymers. When classification is based on their polar groupings most of the biosurfactants are either anionic or neutral and the hydrophobic moiety is based on long chain fatty acids or fatty acids derivatives whereas the hydrophilic portion can be carbohydrates, amino acid, phosphate or cyclic peptide (Nitschke and Coast, 2007). Desai and Banat,(1997) stated that in general, the structure of a biosurfactants includes a hydrophilic moiety consisting of amino acids or peptides anions or cations, mono-, di- or polysaccharides and a hydrophobic moiety consisting of unsaturated, saturated or fatty acids derivatives.

2. Classification based on Chemical Structure

Glycolipids: Most known biosurfactants are glycolipids, they are carbohydrates whose constituent mono-, di-, tri and tetrasaccharides include glucose, mannose, galactose, rhamnose, galactose sulphate and glucoronic acid. These carbohydrates combine with long–chain 3 Okoliegbe and Agarry 17 aliphatic acids or hydroxyaliphatic acids. This linkage is by means of either ether or an ester group. In this taxa, the best known are the rhamnolipids, trehalolipids and sophorolipids (Desai and Banat, 1997, Muthusamy *et al*., 2008). Other glycolipids produced by microorganisms are such as cellobiolipids (Desai and Banat, 1997, Muthusamy *et al*., 2008).

Lipopeptides and Lipoproteins: A large number of cyclic lipopeptides including decapeptide antibiotics (gramicidin) and Lipopeptide antibiotics (polymxin) produced by bacteria, *Bacillus brevis* and *Bacillus polymyxa* that had remarkable surface active properties. It is consist of a lipid attached to a polypeptide chain (Desai and Banat, 1997, Muthusamy *et al*., 2008).

Fatty Acids, phospholipids and neutral lipids: several bacteria and yeast produces large quantities of fatty acids and phospholipids type of surfactant during growth on n-alkanes substrate. Fatty acids produced from alkanes are as a result of microbial oxidations that have been considered as surfactant. Apart from these straight chain acids, micro-organisms produce complex fatty acids containing OH groups and alkyl branches, example of such complex acids include corynomuolic acids (Rahman and Gakpe, 2008).

Phospholipids: Phospholipids are major components of microbial membranes, when certain hydrocarbon degrading bacteria or yeast are grown on alkane substrates the level of the phospholipids increases greatly. When bacteria *Acinetobacter* sp HOI-N grown on Hexadecane substrate it produces the phospholipids that is mainly phosphatidylethanolamine (Muthusamy *et al*., 2008).

Polymeric microbial surfactants: most of these biosurfactants are polymeric heterosaccharide containing proteins. The best studied polymeric biosurfactants are emulsan, liposan, mannoprotein and polysaccharide protein complexes (Desai and Banat, 1997).

Particulate Biosurfactant: there are some bacteria that produce extracellular membrane vesicles partition hydrocarbons that form micro emulsion. The microemulsions formed by them play an essential role in uptake of alkane by microbial cells for eg in case of *Acinetobacter* sp (Desai and Banat, 1997).

Properties of biosurfactant

a. It reduces the surface tension of water.
b. They have excellent capacity of forming critical micelle concentration (CMC).
c. The lethality of biosurfactants is very low.
d. They have good compatibility and digestibility.

Methods used to screen biosurfactant efficiency (Amalesh *et al*., 2012**) :** For the screening of biosurfactant efficiency different methods were used such as Hemolysis test, Oil spreading test, Drop collapse method, Emulsification index, hydrocarbon overlay agar method and Blue agar plate method.

1. **Hemolytic activity**: The isolated strain was streaked on the blood agar plate and plate was incubated at 37^0C for 48 to 72 h. After incubation halo zone around the spotted colonies indicate positive result. Zone is classified as alpha, beta and gamma.α-hemolysis was observed when spotted colony gave greenish zone around its inoculation, β-hemolysis was observed when a clear white zone was observed around the inoculated colony and γ-hemolysis was recorded when there was no change around the spotted colony.
2. **Drop collapsing test**: Drop collapsing test is the qualitative process useful for the screening of biosurfactants. The isolated strains were placed on the surface of hydrocarbon. The destabilizations of cell free broth drop indicate positive result. A drop of water acted as a control.
3. **Oil spreading test**: On empty petri plate two different layers were formed. First layer would be of water and second layer would be of hydrocarbon. The 24 hrs old cell free extract broth of isolate was added surface on petri plate. The clear zone around the culture indicates positive result. The diameter of the clear zone was measured. A drop of water acted as a control.
4. **Emulsification index test**: Emulsification index is the quantitative process. In test tubes 2mL of hydrocarbon was added along with 2mL of 48 hrs grown culture broth. It was further vortex for 2 min and allowed to stand by 24 hrs. After 24 hrs of incubation emulsification index was calculated according to standard methodologies.
5. **Blue agar plate or CTAB agar plate method**: This process is used to detect production of extracellular glycolipids. CTAB agar plate containing Methylene blue (5mg/mL) and cetyltrimethylammonium bromide (CTAB) (0.2mg/mL) was prepared. 24 hrs old bacterial isolate was spot inoculated on it and incubated for 24 to 48 hrs. The formations of dark blue

halos around the spotted colonies indicate positive result.

6. **Hydrocarbon overlay agar method**: Hydrocarbon coated LB agar plate was inoculated with 24 hrs inoculated broth. The plate was incubated at 37^0C for 48 to 72 hrs. After inoculation the growth was observed on LB plate. A colony surrounded by the emulsified halos was considered positive for biosurfactant production.

Significance of Biosurfactant to Microbial Cell

Many microbes are slow growers. When slow growers produce emulsifiers it has selective advantage to them as compared to fast growers. It is normally produced by bacteria when they are in stationary phase of growth. It releases chemical signals that are known as quorum sensing. These signals are secreted extracellular or in with attached to parts of cells predominately on water immiscible substrates. The production of biosurfactants by microbial cells plays an important role in increasing their nutrient availability of the substrates. Biosurfactants also have antimicrobial activity that allows them to increase their cell biomass. An organism produces different types of biosurfactants that have wide applications in field of petroleum as described in table-1.

Table-1: Microbes involved in bioremediation (Sillva *et al*., 2014)

Type of biosurfactant	Application	Microorganism
Glucolipid and trehalose lipid	Oil spill cleanup operations	*Rhodococcus erythropolis 3C-9*
Trehalose tetra ester	Bioremediation of oil-contaminated Environments	*Micrococcus luteus* BN56
Lipopeptide	Bioremediation of marine oil pollution	*Rhodococcus* sp. TW53
	Environmental applications	*Azotobacter chroococcum*
	Bioremediation of hydrocarbon-contaminated sites	*Bacillus subtilis* BS5
	Bioremediation	*Nocardiopsis alba* MSA10
Rhamnolipid	Bioremediation of oil-contaminated sites	*Pseudomonas aeruginosa* S2
	Bioremediation of hydrocarbon-contaminated sites	*Pseudomonas aeruginosa* BS20
	Environmental applications	*Pseudoxanthomonas* sp. PNK-04
	Environmental applications	*Pseudomonas alcaligenes*
	Bioremediation of marine and soil environments	*Pseudomonas cepacia* CCT6659
Sophorolipids	Environmental applications	*Candida bombicola*
	Oil recovery	*C. lipolytica* UCP0988
	Control of environmental oil pollution	
	Removal of petroleum and motor oil	
Glycolipid	Bioremediation applications	*R. wratislaviensis* BN38
	Bioremediation of marine oil pollution	*Pseudozyma hubeiensis*
	Bioremediation of marine environment	*Nocardiopsis lucentensis* MSA04
	Removal of petroleum derivate motor oil from sand	*C. guilliermondii* UCP0992
Protein-carboydrate-lipid Complex	Oil recovery from sand	*C. glabrata* UCP1002
	Bioremediation processes	*C. sphaerica* UCP0995
	Oil removal	*C. glabrata* UCP1002
	Removal of petroleum and motor oil adsorbed to sand	*C. tropicalis* UCP0996
Mannosylerythritol lipid	Oil removal	*C. sphaerica* UCP0995
	Bioremediation of marine environment	*Calyptogena soyoae*

Relevance of Biosurfactants in Other Areas

The worldwide production of biosurfactant has increased tremendously. There is an increase in production of biosurfactants @ 3 to 4 % per year globally. The individual applications of surfactants are classified according their relevance to the particular field.

1. **In Agriculture**: In agriculture, surface active compounds are used for hydrophilization of heavy soils to sustain its nutrient ability.

2. **In Medicine**: In medicine they have various applications.
    a. Antimicrobial activity:(Gharaei-Fathabad, 2011).
    b. Antiviral activity: (Desai and Banat, 1997., Muthusamy *et al.*, 2008).
    c. Anti-cancer activity: (Muthusamy *et al.*, 2008).
    d. Anti-adhesive agents: (Rodrigues *et al*., (2006).
    e. Adjuvants in Immunology: (Gharaei-Fathabad, 2011).
    f. Gene delivery: (Muthusamy *et al.*, 2008).
3. **In Industry**: The surfactants are used as emulsifiers, foaming agents, solubilizers, wetting agents, cleansers and antimicrobial agents. They are also mediators of enzyme action and repellants for insects (Gharaei-Fathabad, 2011).
    a. In-Petroleum: Degradation of hydrocarbon with in oil reservoir enzyme is made possible by it. They act as a biocatalyst for up-grading of petroleum (Perfumo *et al.*, 2010). They enhance oil recovery process by microbes which play an important role in Clean-up of oil containers/ storage tanks and formation of products from petrochemicals (Perfumo *et al.*, 2010).

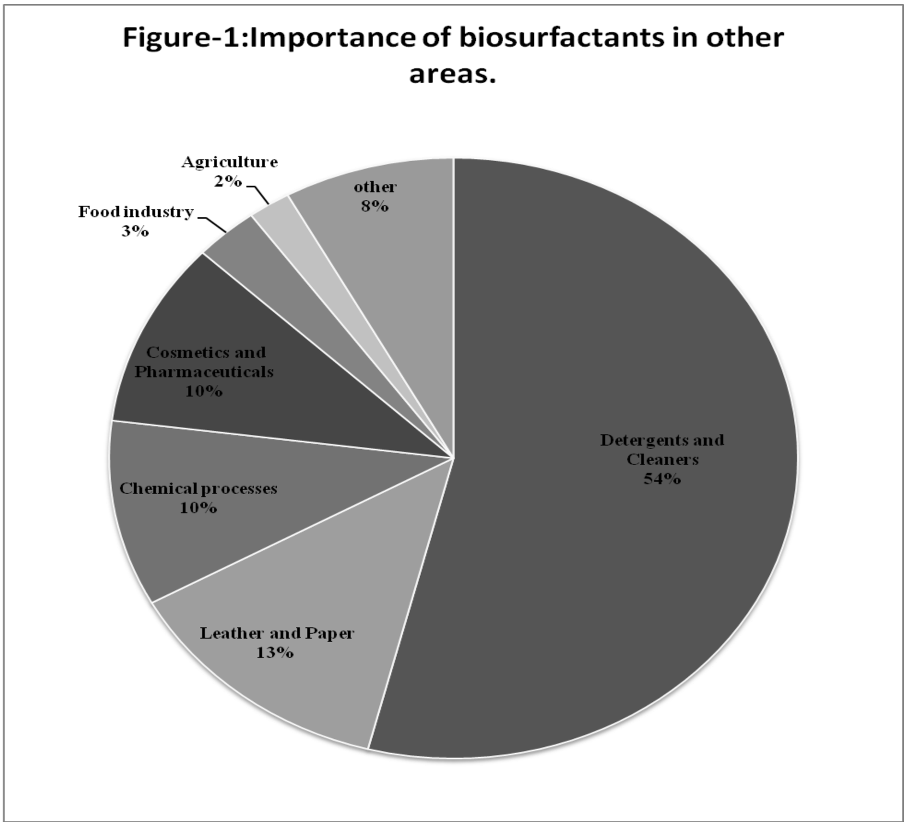

Fig. 1: Describes importance of biosurfactants in other areas (Rahman and Gakpe, 2008)

Advantage of biosurfactants: Biosurfactants have many advantages when compared to chemically synthesized counterparts such as

1. They are easily degraded by microbes.
2. It has low toxicity.
3. It has good compatibility and digestibility with other living organisms.
4. They can be produced from cheap raw materials that are easily available in large quantities.
5. It exhibits emulsification capacity.
6. They are ecologically accepted due to their property of maintaining sustainability (Fig. 2).

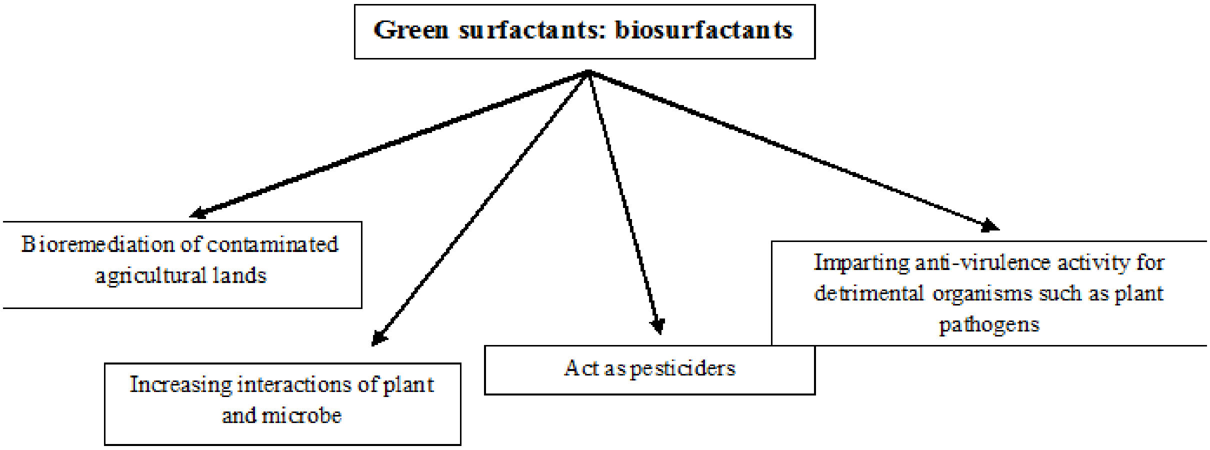

Fig. 2: Biosurfactant acceptance as green surfactants and their Importance in maintaining sustainability.

Conclusion

Biologically synthesized surface active agents are known as biosurfactants. Many methods are there to check the biosurfactants potentiality of the bacterial isolates such as Hemolytic activity, Drop collapsing test, Oil spreading test, Emulsification index test, Blue agar plate or CTAB agar plate method and Hydrocarbon overlay agar method. In our study we found drop collapse and hydrocarbon overy agar method to be most sensitive for detecting biosurfactant production. These biosurfactants have importance in various industries such as in chemical, cosmetic, pharmaceutical, agricultural and food. Its impact is highlighted when it governs a property of emulsification. Due to emulsification microbes enhance the pseudosolubilization of any immense fraction of the contaminants to its minimum concentrations. Many strategies have been studied and developed to decontaminate and restore polluted sites till date. The use of biosurfactants is presented as a best alternate option because of its versatility, biodegradability and ecofriendly nature that maintains sustainability of the environment in a much desired way.

Conflicts of Interested: None

Source of Support: Nil

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How to cite this article: Shah N, Nikam R, Gaikwad S, Sapre V, Kaur J. Biosurfactant: Types, Detection Methods, Importance and Applications. Indian J Microbiol Res 2016;3(1):5-10.