An Overview of the Biosurfactants Enhanced Bioremediation of Hydrocarbons

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Abstract: ***The alarming rate of oil spills is escalating by each passing day. Oil spills have affected humans, marine animals and birds. These spills expand in the mean time and become an alarming threat for various lives present surrounding that particular ecosystem. The contamination causes skin irritation, respiratory disorders, and hereditary diseases in case of humans whereas the oil coats the feathers of the birds and causes distorting issues in flying. The reproduction and nesting time of these birds also get affected. Similarly, the delicate relationships of marine organisms get disrupted and the food gets contaminated. Biosurfactants are the surface active biomolecules present in microorganisms. In recent years, biosurfactants have earned a great attention as they hold the potential of emulsifying and biodegrade the crude oil. This study aims to address the types, properties, mechanism of action and production of biosurfactants from microorganisms.***

Keywords: Biosurfactants, Bioremediation, green method to hydrocarbon degradation

1. Introduction

The New York Times hits the headlines on 8th July, 2020 as follows, *"Mauritius Faces Environmental Crisis as Oil Spills From Grounded Ship; A Japanese-owned bulk carrier ran aground near the Indian Ocean island in late July, with nearly 4,000 tons of fuel oil and 200 tons of diesel on board. And now its hull has cracked."* The average number of annual oil spills is more than 700 tons in between 1970 to 2019, each decade worldwide. Crude oil is a naturally occurring substance in the environment. But over past few years, more oil has escaped into the environment than collected under the ground. The rate at which these degradation processes takes place is affected by factors, for instance, light intensity, oil thickness, aeration and nutrients available *[Kingston et al.2002]*. Heavy oil spills such as Mauritius oil spill may persist for a length of time which can considerably affect the tissues of the marine organisms as well as disturb the marine ecosystem. The compounds from the BTEX group, present in gasoline and diesel, present a higher solubility in water than the other components of these fuels, which explains the fact that, when an underground tank leaks, they are the contaminants found in larger amounts on the water table, being led by groundwater *[Souza et al.2014]*

As the technology is advancing so is the need for more biodegrading microorganisms. This has led to a conclusion that most of them are of natural origins being part of several biological membranes and cellular structures. *[Souza et al.2014]*Most of these surfactants are synthesized by living organisms, such as: saponins, produced by plants; glycolipids, by microorganisms; and bile salts, by animals. These compounds with surfactant properties produced by microorganisms are called biosurfactants. *[Aparna et al.2012]* Biosurfactants are molecules that have both hydrophobic and hydrophilic properties and are able to lower the surface tension and the interfacial tension of the growth medium. They possess different chemical structures—lipopeptides, glycolipids, neutral lipids and fatty acids.*[Cameotra et al. 2010]* Biosurfactants are biodegradable as well as non toxic. These also exhibit strong emulsification of hydrophobic compounds. Low water solubility of these hydrophobic compounds limits their availability to microorganisms, which is a potential problem for bioremediation of contaminated sites. Therefore, biosurfactant-enhanced solubility of pollutants has potential applications in bioremediation. *[Cameotra et al. 2010]* Not only are the biosurfactants useful in a variety of industrial processes, they are also of vital importance to the microbes in defense strategy, bioavailability, emulsification, adhesion and desorption.

Table 1: Types of modern surfactants used in industries

Surfactant types	Examples	% of total production	Major uses
Anionic	Carboxylates, Sulphonates, Sulphuric acid	66	Washing Powders
Cationic	Amine oxides, monoamines, quaternary ammonium salts	9	Fabric softner shampoos
Non- ionic	Carboxylic acids and carbohydrate, esterd, glycerides and their ehoxylated derivatives	24	Laundry cosurfactants, washing up liquids, Personal care products and food
Amphoteric	Alkyl betaines, alkyl dimethylamines, Imidazonilinum derivatives	~1	Speciality use

Adapted from Banat et al (2000) and Desai and Banat (1997).

2. Properties of Biosurfactants

Various properties of microbial surfactants are as follows;

1) **Biodegradability:** Microbes derived products are way easy to degrade as compared to the synthetic surfactants and are not harmful for the environment. Whereas the synthetic surfactant posess a threat to the environment, biosurfactant not only Polyaromatic hydrocarbons but phenanthrene from the aquatic ecosystem. *Lee et al. (2008)* controlled the algal blooms of marine algae,

Cochlodinium using the biodegradable surfactant by using Sophorolipid with 90% removal in 30 minutes.

2) **Surface and Interface activity:** Surfactants reduce the surface tension and interfacial activity. B. subtilis reduces the surface tension to 25 mNm-1 and Pseudomonas to 26mNm-1 whereas the interfacial tension to 1mNm-1. The Critical Micelle concentration of biosurfactants is less than chemical surfactants which means for maximal decrease in surface tension, less surfactant is needed.*[Vijayakumar et al. 2015]*
3) **Temperature and pH tolerance:** Most of the biosurfactants and their surface activity are resistant to temperature and pH. *McInerney et al*. (1990) reported that lichenysin from *Bacillus licheniformis* was found to be resistant to temperature up to 50°C, pH between 4.5 and 9.0 and NaCl and Ca concentrations up to 50 and 25 g L^{-1}, respectively. On the other hand, biosurfactant produced by *Arthrobacter protophormiae* was found to be both thermostable (30-100°C) and pH (2 to 12) stable *(Singh and Cameotra, 2004*). Since, industrial processes involve exposure to extreme temperature, pH and pressure, it is necessary to isolate novel microbial products that able to function under these conditions (*Cameotra and Makkar, 2004).*
4) **Low Toxicity:** Due to the low toxicity, the biosurfactants are considered to be used for pharmaceuticals, cosmetics and food industries. *Poremba et al*. (1991) demonstrated the higher toxicity of the chemical-derived surfactant (Corexit) which displayed a LC50 against *Photobacterium phosphoreum* and was found to be 10 times lower than of rhamnolipids. *Flasz et al*. (1998) compared the toxicity and mutagenicity profile of biosurfactant from *Pseudomonas aeruginosa* and chemically derived surfactants and indicated the biosurfactant as non-toxic and non-mutagenic. The low toxicity profile of biosurfactant, sophorolipids from *Candida bombicola* made them useful in food industries *(Cavalero and Cooper, 2003).*
5) **Emulsion forming and emulsion breaking:** Biosurfactants may act as emulsifiers or de-emulsifiers. An emulsion is a heterogeneous system, consisting of one immiscible liquid dispersed in another in the form of droplets, whose diameter in general exceeds 0.1 mm. Emulsions are of two types: oil-in-water (o/w) or water-in-oil (w/o) emulsions. Their minimal stability can be stabilized by additives such as biosurfactants and can be maintained as stable emulsions for months to years (*Velikonja and Kosaric, 1993).* Liposan, a water-soluble emulsifier synthesized by *Candida lipolytica* , have been used to emulsify edible oils by coating droplets of oil, thus forming stable emulsions. These liposans were commonly used in cosmetics and food industries for making oil/water emulsions for making stable emulsions *(Cirigliano and Carman, 1985).*
6) **Antiadhesive agents:** A biofilm can be described as a group of bacteria/other **organic matter** that have colonized/accumulated on any surface (*Hood and Zottola, 1995).* The first step on biofilm establishment is bacterial adherence over the surface was affected by various factors including type of microorganism, hydrophobicity and electrical charges of surface, environmental conditions and ability of microorganisms to produce extracellular polymers that help cells to anchor to surfaces *(Zottola, 1994).* The biosurfactants can be used in changing the hydrophobicity of the surface which in turn affects the adhesion of microbes over the surface. A surfactant from *Streptococcus thermophilus* slows down the colonization of other thermophilic strains of *Streptococcus* over the steel which are responsible for fouling. Similarly, a biosurfactant from *Pseudomonas fluorescens* inhibited the attachment of *Listeria monocytogenes* onto steel surface *(Chakrabarti, 2012).*

3. Mechanism of Action of Biosurfactants

There are two ways behind the mechanism of action of biosurfactants

1) Increasing the surface area of hydrophobic water insoluble substrates:

The bacteria growing on hydrocarbons have the very limited growth because of the interfacial surface area between water and oil (Sekelsky and Shreve, 1999). When the surface area becomes limiting factor, biomass increases arithmetically rather than exponentially. Generally, emulsification is a cell-density-dependent phenomenon: that is, greater the number of cells, the higher the concentration of extracellular product. The concentration of cells in an open system, such as an oil-polluted body of water, never reaches a high enough value to effectively emulsify oil. Furthermore, any emulsified oil would disperse in the water and not be more available to the emulsifier-producing strain than to competing microorganisms.

One way to reconcile the existing data is that the emulsifying agents do play a natural role in oil degradation but not in producing macroscopic emulsions in the bulk liquid. If emulsion occurs at, or very close to, the cell surface and no mixing occurs at the microscopic level, then each cluster of cells creates its own microenvironment and no overall cell density dependence would be expected.

2) **Increasing the bioavailability of hydrophobic water-insoluble substrates:** The low water solubility of many hydrocarbons, specially the Polycyclic Aromatic Hydrocarbons (PAHs), is believed to limit their availability to microorganisms which is a potential problem for bioremediation of contaminated sites. It has been an assumption that surfactants would enhance the **bioavailability** of hydrophobic compounds. Several non-biological surfactants have been studied and both good and bad effects of the surfactants on biodegradation were observed. For instance, the addition of the surfactant Tergitol NP-10 increased the dissolution rate of solid-phase phenanthrene and resulted in an overall increase in the growth of a strain of *Pseudomonas stutzeri* (*Grimberg et al., 1996).* A similar effect was obtained by the addition of Tween 80 to two *Sphingomonas* strains, the rate of fluoranthene mineralization was almost doubled. By contrast, the same surfactant inhibited the rate of fluoranthene mineralization by two strains of *Mycobacterium* (*Willumsen et al., 2001)* and no stimulation was

observed in other studies using several surfactants *(Bruheim and Eimhjellen, 1998).*

4. Methods for the Production of Biosurfactants

In this experiment, the sample is being collected from six locations that are in Elefsina bay, Attica and Aegan Sea. *(Antoniou et al. 2015)* Seawater and sediment samples were collected from the six locations in Elefsina bay, Attica, Aegean Sea. Elefsina bay is an industrial area, where there are two large petroleum refineries. Due to several accidents in the past and the slow seepage of CO from old storage tanks, there is sufficient evidence of low chronic pollution in the area. The sampling campaign aimed to isolate consortia and strains from both the water column and the sediment which enhances the probability to isolate different strains of interest. The samples were collected downstream of the local current direction (West-to-East).

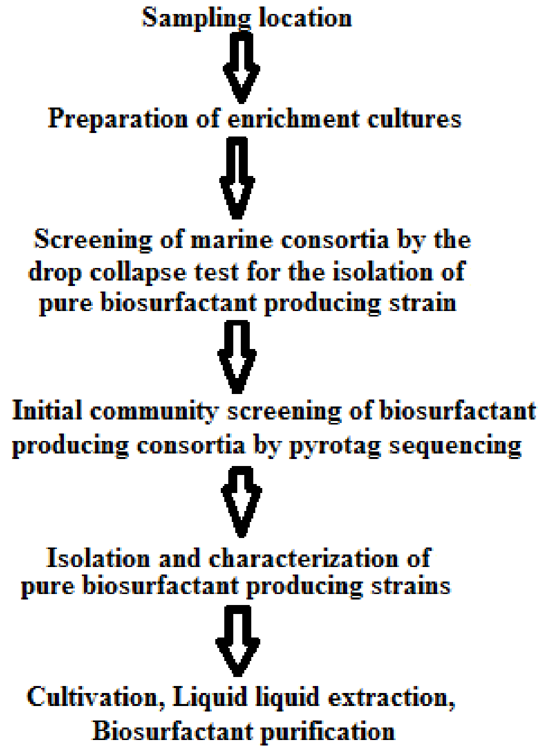

Figure 1: Materials and methods needed for isolation of pure biosurfactant producing strain

Biosurfactant detection characterization can be done by Thin layer chromatography, Fourier Transform Infrared spectroscopy and Mass spectroscopy. *(Antoniou et al. 2015)*

Table 2: Species of microorganisms producing different types of biosurfactants and their uses

MICROORGANISM	TYPE OF BIOSURFACTANT	USES
1. *Rhodococcus erythropolis 3C-9*	Glycolipid, Trehalose lipid	Oil spill cleaning operations
2. *Pseudomonas aeruginosa S2*	Rhamnolipid	Bioremediation of places contaminated with petroleum
3.*Pseudomonas aeruginosa CBS9960*	Mannosylerythritol lipid	Promising yeast biosurfactant
4. *Pseudozyma graminicola CBS 10092*	Mannosylerythritol lipid	Washing detergent
5. *Pseudomonas libanesis M9-3*	Lipopeptide	Environmental and biomedical uses
6. *Bacillus subtilis ZW-3*	Lipopeptide	Pharmaceuticals, environmental protection, cosmetics and petroleum recovery
7. *Rhodococcus sp. TW 53*	Lipopeptide	Bioremediation in sea environment
8.*Pseudozyma hubeiensis*	Glycolipid	Bioremediation in sea environment
9.*R. wratislaviensis BN 38*	Glycolipid	Bioremediation uses
10.*Bacillus subtilis BSS*	Lipopeptide	Bioremediation of places contaminated by hydrocarbon
11.*Azotobacter chrococcum*	Lipopeptide	Environmental uses
12. *Pseudomonas aeruginosa BS20*	Rhamnolipid	Bioremediation of places contaminated by hydrocarbon

13.*Micrococcus luteus BN56*	Trehalose tetraester	Bioremediation contaminated by petroleum
14.*Bacillus subtilis HOB2*	Lipopeptide	Petroleum recovery, bioremediation of soil and sea environment and food industry
15. *P. aeruginosa UFPEDA614*	Rhamnolipid	Bioremediation
16. *Nocardiopsis alba MSA10*	Lipopeptide	Bioremediation
17.*Pseudoxanthomonas sp. PNK-04*	Rhamnolipid	Environmental uses
18.*Pseudozyma parantarctica*	Mannosylmsnnitol lipid	Detergent or washing emulsification
19. *Pseudomonas alcaligenes*	Rhamnolipid	Environmental uses
21.*Pseudomonas kareensis*	Lipopeptide	Biological control agent
22. *Pseudomonas fluorescens BDS*	Lipopeptide	Bioremediation and Biomedicine
23. *Candida bombicola*	Sorolipideos	Environmental uses
24.*Brevibacterium aureum MSA 13*	Lipopeptide	Petroleum recovery
25. *Nocardiopsis lucentencis MSA04*	Glycolipid	Bioremediation in sea environment
26. *Bacillus velenzensis H3*	Mannosylerythritol lipid	Bioremediation in sea environment

5. Conclusion

Environment has been severely affected, the reason being the pollutants which include oil spills as well. The Mauritius oil spil, the Gulf of Mexico, The Odyssey, The Amoco cardiz are the names of very few. The oil pollutants have disturbed the nature's balance. We can even even notice those altering climate changes as well. It has even contaminated even the air that we breath in. Rather than using the chemical surfactant that only excurbate the situation, we need to find more of naturally originated surfactants or biosurfactants. Microbes hold the potential capacity to be a cure for everything. Biosurfactants, not only save the environment but also add up all the goods into the soil, air and water. These biosurfactants can be developed on a lab scale with the aid few equipments which makes it a cost effective method. But still we need to produce biosurfactants on large scale which seems to be a challenge right now. Another challenge that is being faced in the production of biosurfactants is that very few microorganisms or no microorganism is there that can completely degrade the crude oil, the reason being the interfacial tension. Therefore, the types of microorganisms that can actually completely degrade the crude oil are needed to be discovered. Moreover, biosurfactants are not only used for bioremediation but can be used in food industries, pharmaceuticals and cosmetic industry which makes it more profitable. But unfortunately more research is needed in the field. In the future trends, biosurfactants could become the solution for oil spills.

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