

**BIOSURFACTANT: AN ENVIRONMENT FRIENDLY SUBSTITUTE  
TO SURFACTANT****Zalak N. Patel and N. Saraswathy\***Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's  
NMIMS, Mumbai, India.Article Received on  
09 December 2013  
Revised on 06 January 2013,  
Accepted on 10 February  
2014**\*Correspondence for****Author:****N. Saraswathy,**  
Shobhaben Pratapbhai Patel  
School of Pharmacy &  
Technology Management,  
SVKM's NMIMS, Mumbai,  
India.**ABSTRACT**

Biosurfactant are surface active compounds involved in reduction of surface tension between oil and water phase. They are generally synthesised from micro-organisms. The aim of this review article is to give a brief introduction of biosurfactant, its mechanism of working and various strains of micro-organism producing biosurfactant. There are many advantages of biosurfactant over chemical surfactant. This article describes in brief the present scenario of the oil spillage incidents all round the worlds. Bioremediation is one of the best applications which can prove to be helpful in dealing with this oil spills and make biosurfactant an environment friendly substitute to surfactant.

**Keywords:** Biosurfactant, Microorganism, Bioremediation, Environment.

**INTRODUCTION**

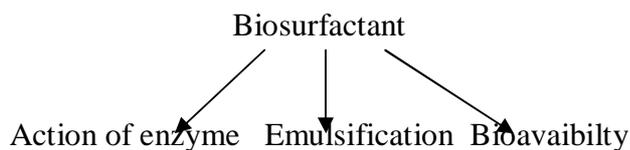
Surfactants are usually organic compounds containing both hydrophobic group and hydrophilic group<sup>(1)</sup>. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Surfactants are classified as anionic, cationic, zwitterionic and non-ionic surfactants<sup>(2)</sup>. A surfactant is amphiphilic compound so it contains both a water soluble (or oil soluble) component and a water soluble component. Surfactants generally gets diffuse in water and absorb at the interface between the oil and water<sup>(28)</sup>. The insoluble hydrophobic group may extend out of the bulk water phase, into the oil phase, while the water soluble head group remains in the water. Thus such an alignment of surfactant at interface of oil and water layer modifies the surface property of water in water/air or water/oil interface<sup>(3)</sup>. Surfactants play an important role

as cleaning, wetting, dispersing, emulsifying, foaming and anti-foaming agents in various process and products like detergents, emulsions, paints etc<sup>(3)</sup>.

The day to day increase in use of surfactant in almost all routine and household purposes has lead to deposition of surfactant on land, oceans, rivers etc. These deposited surfactants were found to be toxic and harmful to various living creature. Thus there arose a need of biosurfactant which can also be called as environment friendly surfactant.

Biosurfactants are surface active substances synthesized by living cells; they are generally non-toxic and biodegradable<sup>(28)</sup>. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions, and potential applications in environmental protection<sup>(28)</sup>. Commercially very limited types of biosurfactants are present. Thus there is a need of producing new variety of efficient biosurfactant. There are various criteria for production of biosurfactant. 1) to find new structures with strong interfacial activity, low critical micelle concentration, high emulsion capacity, good solubility and activity in broad pH range. 2) To produce an economically competitive biosurfactant i.e to find out a good production strains with high yields. 3) to produce biosurfactant from non-pathogenic strains so that they can act as environment friendly surfactant<sup>(21)</sup>.

#### MECHANISM OF OIL DEGRADATION BY BIOSURFACTANT:



##### 1) Action of enzyme:

The hydrocarbon degradation involves a membrane bound oxygenase, makes it essential for bacteria to come in direct contact with the hydrocarbon substrates<sup>(20)</sup>.

##### 2) Emulsification:

The hydrocarbon degradation process can be enhance by increasing contact between bacteria and hydrocarbon by emulsification of hydrocarbon. The surface area of the hydrophobic water-insoluble substrates is increased by emulsification<sup>(20)</sup>.

##### 3) Increase in bioavailability

Biosurfactant can enhance growth on bound substrates by desorbing them from surfaces or by increasing their apparent solubility. Thus they increase the bioavailability of water-insoluble substrates to bacteria. surfactants that lower the interfacial tension to greater extend are particularly

effective in mobilizing bound hydrocarbon. Low molecular weight biosurfactants have low critical micelle concentration increase the apparent solubility of hydrocarbons by incorporating them into hydrophobic cavities of micelles<sup>(20)</sup>.

### SOURCES OF BIOSURFACTANT

There are various sources from which biosurfactants are produced. Biosurfactants can be classified on basis of its source/origin. Following are the three broad class of biosurfactant<sup>(22)</sup>:

- 1) Microbially derived
  - 2) Animal derived
  - 3) Plant derived
- 1) Microbially derived<sup>(22)</sup>

Microbially derived biosurfactants are surface active agents derived from yeasts, bacteria and fungi. They vary in molecular weight. They are divided as low molecular weight and high molecular weight. Lowmolecular weight biosurfactants include glycolipid, phospholipid and lipopeptide. They are effective in reducing interfacial tension. High molecular weight biosurfactant include lipopolysaccharides, proteins, polysaccharides, lipoproteins and complex mixture of biopolymers. High molecular weight biosurfactants are effective in stabilizing newly created surface. They are generally used in food, cosmetics and pharmaceutical industry.

#### 2) Animal derived<sup>(22)</sup>

There are various type of animal derived biosurfactant such as lecithin, gelatine, casein, wool fat, cholesterol and wax.

Gelatine is obtained by partial hydrolysis using dilute acid or base. Commercially obtained from bovine skin and bones and pigskin. Gelatine is a high molecular weight biosurfactant mainly used as thickener, stabilizer

Lecithin is low molecular weight surfactant obtained from egg yolk. Purified egg lecithin is used as main excipient in pharmaceutical industry for drug.

Casein is a milk protein that accounts for approximately 80 % of milk's total protein. Casein can be prepared by isoelectric precipitation or enzyme precipitation. Casein are widely used as emulsifiers, thickeners and gelling agent in various food products.

Whey protein is a mixer of globular protein and milk protein. Its emulsifying properties are greatly affected by ph, ionic strength and temperature. Egg albumin, bovine serum albumin and human serum albumin are other widely used protein based biosurfactants from animal origin.

Pulmonary surfactant is a complex mixture of lipids and proteins that coats the interior surface of vertebrate lung as a film. Pulmonary surfactant mainly consists of phospholipids. Pulmonary surfactant is used as exogenous surfactant replacement therapy in case of deficiency or inadequate function of normal pulmonary surfactant which can lead to respiratory disorder. They are obtained as synthetic or from the bovine or porcine sources.

There are lot of disadvantages of animal derived biosurfactants i.e. high stock of animal feedstock, variability in product from batch to batch, religious restrictions etc. Thus plant derived biosurfactant are more preferred the animal derived biosurfactant.

### 3) Plant derived<sup>(22)</sup>

Saponins are derived from various parts of plants like leaves, root, stem, etc. The most significant are dietary legumes like soybeans, chickpeas, mung beans, peanuts and lentils. Saponins have emulsifying and foaming property, pharmacological and medicinal properties and are used in beverages, cosmetics and pharmaceutical products.

Lecithin is low molecular biosurfactant obtained from both animal and plants. Lecithin in case plants are obtained from soybeans oilseeds. Soylecithin are widely used as emulsifier, stabilizers, wetting agent, antioxidant, lubricants and nutritional supplements.

### APPLICATION OF BIOSURFACTANT<sup>(23,24)</sup>

1. Accelerated bioremediation of hydrocarbon – contaminated sites.
2. Enhanced oil recovery in case oil spreading in oceans.
3. Herbicides and pesticides formulations, detergents healthcare and cosmetics.
4. Pulp and paper, coal, textiles, ceramic processing and food industries.
5. Uranium ore – processing.
6. Mechanical de-watering of peat.
7. Some biosurfactants also have antimicrobial effects, antiviral effects etc.
8. Removal of oil sludge from oil storage tanks.
9. In petroleum production and incorporation into oil formulations
10. The host of interesting features of biosurfactants have led to a broad range of potential application in the biomedical field.
11. Potential use as major immunomodulatory molecules, anti-adhesive agents and even in vaccines and gene therapy.
12. In petroleum industry they are used in mobilizing heavy crude oil, transporting petroleum in pipelines and stabilizing fuel water-oil emulsions.

**TYPES OF BIOSURFACTANT<sup>(17)</sup>:**

1. Glycolipid
  - Rhamnolipids – *P.aeruginosa*
  - Trehalose – *Rhodococcus* , *Nocardia*, *Corynebacterium*, *Tsukamurella*
  - Sophorose – *Candida* and *Ustilago maydis*
2. Lipopeptides – *Bacillus* sp.
3. Lipoproteins – *Actinoplanes*, *Arthrobacter*, *Pseudomonas* and *Serratia*.
4. Polymeric biosurfactant – *Acinetobacter*, *Halomonas*, *Methanobacterium* and *Sulfolobus*.

**VARIOUS BIOSURFACTANTS PRODUCED FROM MICROBIAL SPECIES****1) Glycolipid**

Glycolipids are further divided into rhamolipid, Trehalose and Sophorose. Rhamolipid is best studied biosurfactant. Rhamnolipid is one type of glycolipid in which one or two molecules of rhamnose are linked to one or two molecules of hydroxydecanoic acid while the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide<sup>(18)</sup>. Rhamnolipid is produced by *pseudomonas aeruginosa*, gram negative bacteria. Rhamnolipid was produced from *pseudomonas aeruginosa* from oil contaminated soil using four different oils as substrate and isolated biosurfactant was identified by using TLC method<sup>(3)</sup>. *Rhodococcus ruber* and *Rhodococcus erythropolis* were found to produce glycolipid type of biosurfactant. This strains of *Rhodococcus* sp. were grown on hydrocarbon as sole carbon source and were found to produce glycolipid<sup>(6)</sup>.

**2) Lipopeptide**

The lipopeptide category of biosurfactant is generally produced by *Bacillus* sp. *Bacillus* sp. Generally produce a cyclic lipopeptide called as surfactin. Surfactin's structure consists of a peptide loop of seven aminoacids and anhydrophobic fatty acid chain thirteen and fifteen carbon long<sup>(18)</sup>. The biosurfactant production by *Bacillus subtilis* isolated from oil contaminated soil using four different oils as substrate was carried out by T.priya and G. Usharani<sup>(5)</sup>. The biosurfactant produced from *Bacillus* sp. was identified using TLC method. There are some articles regarding production of lipopeptide from *Enterobacter cloacae* LK5. Lipopeptide was found to be present in the oil contaminated soil having the species of *Enterobacter cloacae* LK5. Siripun Sarin and Charoon Sarin had conclude that lipopeptide was produced and suggested that the biosurfactant might be useful in oil removal, while the determination on foamability and stabilizing capacity of

biosurfactant/bioemulsifier indicated that bioemulsifier with reducing surface tension property could prove to be a potential compound for emulsion application<sup>(25)</sup>.

### 3) Phospholipids

Phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline. Phospholipid was isolated from soil containing *Sphingobacterium* sp. The TLC identification showed presence of phospholipid in the purified extract of biosurfactant<sup>(19)</sup>.

### 4) Polymeric biosurfactant

Polymeric biosurfactant are generally produced by *Acinetobacter*, *Halomonas*, *Methanobacterium* and *Sulfolobus*. Polymeric biosurfactant are compiled from several components. Emulsan, synthesized from *Acinetobacter calcoaceticus*, is the best example. It consists of a heteropolysaccharide backbone to which fatty acids are covalently linked. (Rosenberg et al., 1988). Another example is liposan, a carbohydrate-protein complex synthesized by the yeast *Yarrowia lipolytica* (Cirigliano and Carman, 1984).

## ADVANTAGES OF BIOSURFACTANT<sup>(10,25,26,27)</sup>

The recent advances in the industrialization and petroleum products have led to the deposition of oils and petroleum products in the rivers, sea, oceans etc. The use of chemical surfactant no doubt reduces the oil deposition but also proved to be highly toxic and non-degradable. Thus biosurfactants are the best alternative to this as they have many advantages over chemical surfactants such as:

1. Biocompatibility: biosurfactant being from the biological/natural origin are found to be more environment friendly.
2. Biodegradability: Chemical surfactants are produced by organic chemical reaction, while biosurfactant are produced by biological processes being excreted as extracellularly by micro-organism. Thus can be easily degraded.
3. Low toxicity: Most of the chemical surfactant are petroleum derivatives having higher range of toxicity.
4. Availability of raw materials: biosurfactants can be produced from cheap raw materials which are available in large quantities.
5. Acceptable production economics: Biosurfactants can also be produced from industrial wastes and by-products. Biosurfactants have ability to be synthesized from renewable feedstock.
6. Environmental management: biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents

7. High foaming ability
  8. Stability: Biosurfactants are found to be stable at high temperature, pH and salt concentration.
  9. Bioremediation: Biosurfactants undergo in-situ bioremediation, is considered as environment friendly because it restores the soil structure, requires less energy input and involves the complete destruction or immobilization of the contaminations rather than their transfer from one environment compartment to other, which occur in chemical/physical treatment process.
- Thus from the above discussion we can state that biosurfactant are environment friendly surfactant compared to chemical surfactant.

### **PRESENT SCENARIO OF OIL SPILLAGE**

There is tremendous increase in the rate of incidents regarding the oil spillage in the oceans all over world. Some of the recent incidents of oil spillage in India and around the world are described below:

1. August 2010, MSC Chitra Khalijia collided off the Mumbai coast spilling about 500 tonnes of oil that reach the shores of Mumbai and Raigad. (Hindustan Times)
2. January 2011, Rupture in ONGC's Mumbai-Uran crude oil pipeline was estimated to have spilled 25,000 barrels of crude oil leaked into the sea.(Hindustan Times)
3. August 2011, MV RAK which carried 60,000 metric tonnes coal, 290 tonnes fuel oil and 50 tonnes diesel, sank near Mumbai (Hindustan Times)
4. October 2011, An oil spill from a stranded cargo ship off New Zealand was said to be the country's worst environment disaster in decades as per government of New Zealand.(BBC news)
5. December 2011, Nigeria's deadly delta, A leak at the bonga field during a transfer of oil to a tanker led to 40,000 barrels spillage in the Atlantic ocean.(BBC news).

### **BIOREMEDIATION**

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention<sup>(31)</sup>. There are a lot of pollutants produced by various industries and day to day routine activities. Some of these pollutants are toxic and carcinogenic too. Thus their accumulation in soil and water may prove to be hazardous and life threatening as in case of water the marine creatures are at danger while in case of soil plants may take up this toxic component which can lead to be hazardous if consumed by humans and animals. Thus various methods are applied to reduce this pollution i.e. undertake bioremediation. The bioremediation of soil can be done using a physical

method by replacing the whole part of soil with the new soil surface but it is not possible for large surface areas<sup>(16)</sup>. Thus the in-situ bioremediation is carried out by addition of the nutrients to the soil which may facilitate the growth of micro-organisms in the soil and this micro-organism may consume the hazardous components and metabolize them into non-toxic product that are safe to the environment<sup>(16)</sup>. The problem in this process is the presence of specific micro-organism which can metabolize the present toxic product. Thus direct addition of the biosurfactant producing strains may decrease the time taken for bioremediation and increase specificity. The biosurfactant produced by these strains can very facilitate the consumption of toxic production of toxic products by the micro-organism<sup>(16)</sup>. The process of bioremediation by biosurfactant is enhanced by two pathways (i) increasing the surface area of hydrophobic water – insoluble substrates (ii) Increasing the bioavailability of hydrophobic water insoluble substrates<sup>(20)</sup>.

## CONCLUSION

The demand of biosurfactants is increasing as they play a crucial role in environmental problem such as oil pollution. Three unique property of biosurfactant such as biodegradability, biocompatibility, and digestibility in comparison to artificial surfactant has its use in decreasing oil pollution. Various types of biosurfactant are produced from different kind microorganism. Thus from above discussion we can suggest that the biosurfactant is an ecofriendly agent.

## REFERENCES

1. Orathai Pornsunthorntawee, Panya Wongpaint, Sumaeth Chavadej, Masahiko Abe, Ratana Rujiravanit. Structural and physiochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil. *Bioresource Technology*, 2008;99: 1589-1595.
2. Alexander T Florence, David Attwood. Surfactants. *Physiochemical principles of pharmacy*. 2006; 4:178-179.
3. John Toedt, Darrell Koza, Kathleen Van Cleef-Toedt. Soaps and Laundry Products. *Chemical composition of everyday products*, 2005; 1-2.
4. Rosenberg, E., Ron, EZ. High and low molecular mass microbial surfactants. *Appl. Microbiol. Biotechnol*, 1999; 52 (2): 154-162.
5. Priya T., Usharani G. Comparative study for biosurfactant production by using *Bacillus subtilis* and *Pseudomonas aeruginosa*. *Botany Research International*, 2009; 2 (4): 284-287.
6. Anandaraj B, Thivakaram P. Isolation and production of biosurfactant producing organism from oil spilled soil. *J. Biosci. Tech.* 2010; (3):120-126.

7. Mohamed Sifour, Houria Ouled-Haddar and Ghazi Munim Aziz. Production of biosurfactant from two *Bacillus* species. Egyptian Journal of Aquatic Research, 2005; (31): 142-148.
8. Flavio Correa Bicca; Leonardo Colombo Fleck; Marco Antonio Zachia Ayub. Production of biosurfactant by hydrocarbon degrading *Rhodococcus ruber* and *Rhodococcus erythropolis*". Rev. Microbiol. 1999; (30):231-236.
9. Rodrigues LR, Teixeira JA, Oliveria R. Low cost fermentive medium for production by probiotic bacteria. Biochemical Engineering Journal. 2006;(32):135-142.
10. Vijayabaskar P., Babinastarlin S., Shankar T., Sivakumar T., Anandapandian KTK . Quantification and Characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121) . Advances in Biological Research, 2011; 5(2): 71-76.
11. Chunhui Liu, Juan Lu, Lili Lu, Yuhong Liu, Fengshan wang, Min Xiao. Isolation, structural characterization and immunological activity of an exopolysaccharide produced by *Bacillus licheniformis* 8-37-0-1 . Bioresource Technology , 2010;(101) : 5528-5533
12. Lakshmipathy Deepika , Krishnan Kannabrian. Biosurfactant and Heavy Metal Resistance Activity of *Streptomyces* spp. Isolated from Saltpan Soil. British Journal of Pharmacology and Toxicology, 2010; 1(1) : 33-39.
13. Sabina Viramontes-Ramos; Martha Cristia Portillo-Ruiz; Maria de Lourdes Ballinas-Casarrubias. Selection of biosurfactant/bioemulsifier-producing bacteria from hydrocarbon-contaminated soil. Appl Biochem Biotechnol., 2010; (8):1444-60.
14. Lowry OH., Rosenbrough NJ., Farr AL., Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 1951; 193, 265-275.
15. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers. P.A. and Smith, F. Anal. Chem., 1956; 26: 350.
16. Surachai Techaoei. Preliminary screening of biosurfactant producing microorganism isolated from hot spring and garages in northern Thailand. KMITL sci. Tech. J. 2007; 7.
17. Borjana K. Tuleva. Biosurfactant Production by a New *Pseudomonas putida* Strain, 2002 : 358.
18. N. Kosaric . Biosurfactants and their application for Soil Bioremediation . Food Technol. Biotechnol., 2001. 39 (4): 295-304.
19. Catherine N Mulligan. Types, production and application of biosurfactant". Proc. Indian Natn Sci Acad. B70. 2004: (1): 31-55.
20. Karanth NGK, Deo PG and Veenanadig NK. Microbial production of biosurfactants and their importance. Curr. Sci., 1999; 77: 116-123.
21. C. Burgos-Diaz, R. Pons, M. J. Espuny, F. J. Aranda, J. A. Teruel, A. Manresa, A. Ortiz, A. M. Marques. Isolation and partial characterization of a biosurfactant mixture produced by

- Sphingobacterium* sp. isolated from soil. Journal of Colloid and interface science ,2011; 361: 195-204
22. Eliora Z Ron and Eugene Rosenberg. Biosurfactants and oil bioremediation. Current opinion in Biotechnology, 2002; 13: 249-252
  23. Vanessa Walter, Christoph Syldatk and Rudolf Hausmann, Screening concepts for the isolation of biosurfactant producing micro-organism .<http://www.ncbi.nlm.nih.gov/books/NBK6189/>.
  24. Qingyi Xu, Mitsutoshi Nakajima, Zengshe Liu Takeo Shiina. Biosurfactants for microbubbles preparation and application. Int. J. Mol. Sci. 2011:(12); 462-475.
  25. Siripun Sarin and Charoon Sarin. Production, Isolation and Application of biosurfactant/bioemulsifier by oil contaminated soil isolate *Enterobacter cloacea* LK5. Naresuan university journal. 2008: 2;113-125.
  26. Ligia R. Rodrigues, Jose A. Teixeira, Henny C. van der Mei, Rosario Oliveira; (2006) "Physiochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53 colloids and surfaces B: Biointerfaces. 2006:(49):78-85.
  27. P. Bharali, S. Das, B.K. Konwar, A.J. Thakur. Cude biosurfactant from thermophilic *Alcaligenes faecalis*: Feasibility in petro-spill bioremediation. International Biodeterioration and biodegradation, 2011: 65; 682-690.
  28. Banat, IM., Makkar, RS., Cameotra, SS. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biotechnol. 200: 53 (5); 495-508.
  29. T. Deepika Lakshmipathy, A.S. Arun Prasad Krishnan Kannabiran. Production of biosurfactant and heavy metal resistance activity of *Streptomyces* Sp. VITDDK3-a novel Halo tolerant Actinomycetes isolated from saltpan soil. Advances in Biological Research, 2010: 4(2);108-115.
  30. Samuel Furse, Bubbles, bubbles, everywhere but not a drop to drink. The lipid chronicles. 2010
  31. V.S. Millioli, E.L.C. Servulo, L.G.S. Sobral, D.D. DE Carvalho. Bioremediation of crude oil-bearing soil: Evaluating the effect of rhamnolipid addition to soil toxicity and to crude oil biodegradation efficiency. Global NEST journal. 2009: (11):181-188.