



A REVIEW OF BIOSURFACTANTS AND THEIR APPLICATION

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ABSTRACT

In the rapidly developing biotechnology sector, biosurfactants are evolving into desirable microbial products. These surface-active compounds are produced by a variety of microorganisms, including bacteria, fungi, and yeast. They outperform chemical surfactants in a number of areas, including biodegradability, toxicity, compatibility with the environment, selectivity, foaming, and perform specific activities under extreme conditions including temperature, pH, and salinity. Numerous microbes, such as *Acinetobacter* species, *Bacillus* species, *Candida antartica*, *Pseudomonas* species, and *Thiobacillus thiooxidans*, create biosurfactants. In the sectors of cosmetics, food processing, medicines, and environmental bioremediation, biosurfactants are important, especially in enhanced oil recovery (EOR) and cleaning up oil spills. The purpose of this review is to provide a comprehensive overview of the classification of biosurfactants, the production of biosurfactants, and its applications in various fields.

Keywords: Biosurfactants, Applications.

INTRODUCTION

The term "surface active agent" is where the word "surfactant" originates. Surfactants are chemical substances that are used to reduce the surface tension (also known as interfacial tension) between various compounds, such as between two liquids, a gas and a liquid, or a liquid and a solid. They are typically organic molecules with both hydrophobic and hydrophilic groups in their heads and tails (Saharan *et al.*, 2011). While the polar moieties can be cationic, anionic, non-ionic, or amphoteric molecules, the non-polar moieties are frequently chains of hydrocarbons. The minimum surfactant concentration necessary to obtain the lowest surface tension is known as the Critical Micelle Concentration (CMC). When amphipathic molecules reach the CMC, they combine with the hydrophilic parts facing the outside and the hydrophobic parts towards the

inside. After reaching the CMC value, further addition of surfactant has no effect on further lowering the surface tension (Sarubbo *et al.*, 2022).

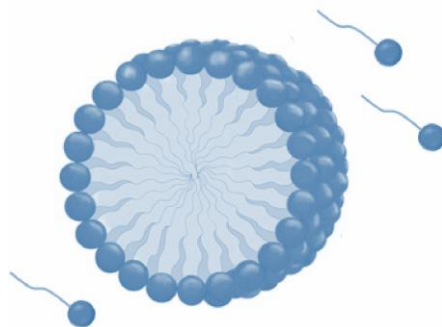


Figure 1: Schematic illustration of micelle formation (Danyelle *et al.*, 2016)

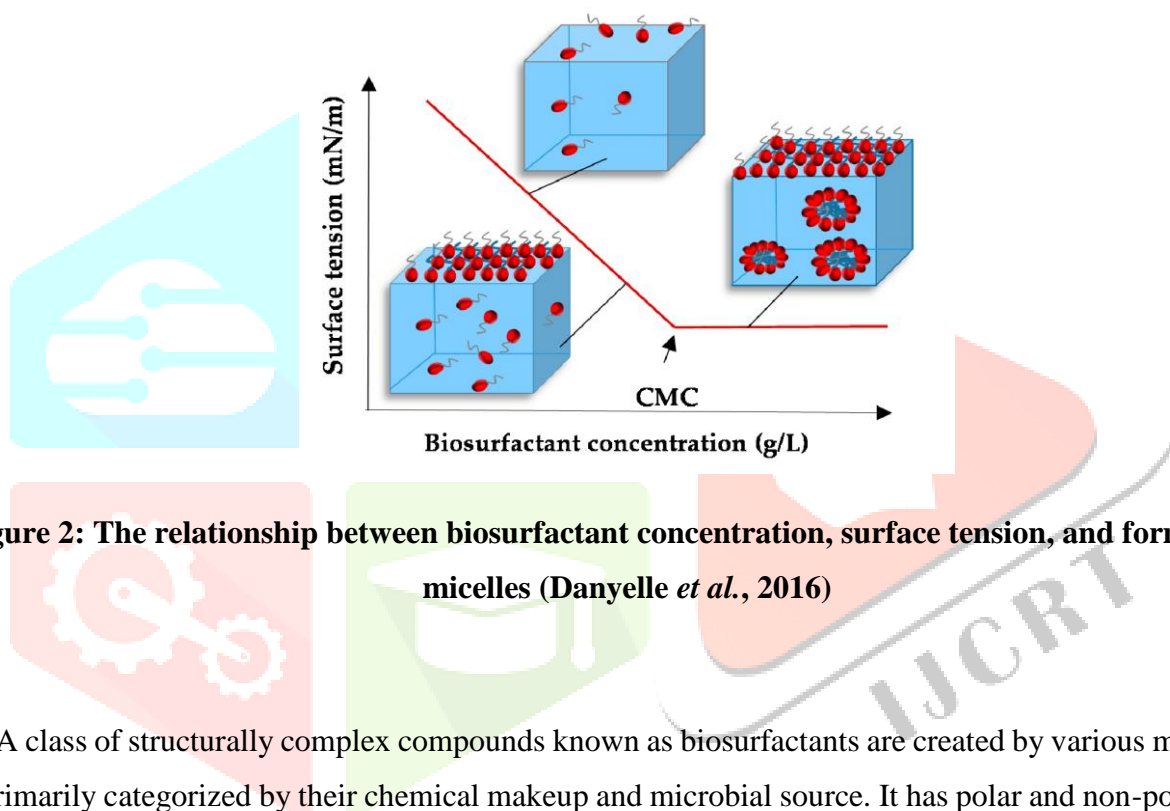


Figure 2: The relationship between biosurfactant concentration, surface tension, and formation of micelles (Danyelle *et al.*, 2016)

A class of structurally complex compounds known as biosurfactants are created by various microbes and are primarily categorized by their chemical makeup and microbial source. It has polar and non-polar moieties that allow it to form micelles at the interface of fluids with different polarity, including water and oil, and they have the ability to lower surface pressure. Different microorganisms deliver biosurfactants. All commercial surfactants are made from non-renewable oil-based materials, which makes them more expensive and poses possible environmental risks. The best solutions to this problem are microbial surfactants.

The earliest studies on microbial biosurfactant production were conducted in the 1960s, and additional studies over the following 60 years have made it possible to manufacture and market a wide range of goods that include these biomolecules (de Almeida *et al.*, 2016). Due to their distinctive qualities in comparison to surfactants generated chemically, these microbial surfactants have lately attracted more scientific interest. They are non-toxic, biodegradable, biocompatible, and effective at low concentrations, which are their distinguishing characteristics. They are more selective and have greater foaming characteristics. They can function under conditions of high salinity, pH, and temperature. They may be made from industrial wastes

and byproducts, which enables affordable manufacturing of biosurfactants and permits using waste substrates while at the same time minimizing their polluting effect.

Commercial production of a number of different kinds of biosurfactants is used in the pharmaceutical and cosmetic industries, while other forms of biosurfactants show promise for use in the food, petroleum, and agricultural sectors. Due to their high surface activity and natural similarity, biosurfactants are frequently used in ecological applications, such as improving oil depletion, acting as cancer preventatives, as antimicrobials in the cosmetics industry, and as antagonistic glues against a few microscopic organisms and yeasts in medical applications (Roy, 2017).

This review paper offers a thorough analysis of the classification of biosurfactants and their application in various fields.

CLASSIFICATION OF BIOSURFACTANTS

The ionic charge within the polar region of the molecule serves as the basis for categorizing surfactants. Based on the polarity of the head group of the molecule, surfactants may be divided into four main categories: anionic, cationic, nonionic, and zwitterionic.

1. Anionic surfactants, which are generated naturally and synthetically and have a negative charge, are the most often used surfactants. They have been used in soaps and personal care products since they are excellent cleaners.
2. Cationic surfactants are utilized as anti-corrosion agents, flotation collectors, hair conditioners, fabric softeners, and bactericides because they work effectively on surfaces having a negative charge.
3. Nonionic surfactants have uncharged hydrophilic head groups and work well at low temperatures as emulsifiers and detergents.
4. Zwitterionic surfactants have poor emulsifying and cleaning abilities and are amphoteric. They are extremely skin-compatible and have great dermatological qualities. As a result, zwitterionic surfactants are employed in producing cosmetics and shampoos.

Biosurfactants can be classified according to their chemical structure and microbial origin. They are classified into two categories by Ron, E. Z., and Rosenberg, E. (2002).; high molecular weight and low molecular weight molecules. Low molecular weight biosurfactants are more effective in reducing the surface tension at the air-water interface and the interfacial tension at the oil-water interface, whereas the higher molecular weight ones are most effectively used in stabilizing oil-in-water emulsions. Low-mass surfactants include glycolipids, lipopeptides, and phospholipids, whereas high-mass surfactants include polymeric and particulate surfactants.

Glycolipids

The majority of known biosurfactants are typically glycolipids in origin. They are carbohydrates that join with long-chain aliphatic acids or hydroxyl fatty acids that connect with either an ester or an ether group. Sophorolipids, trehalolipids, and rhamnolipids are the most well-known glycolipids.

1. Rhamnolipids - Rhamnolipids are amphiphilic molecules that are most commonly made up of 3-hydroxy fatty acids (hydroxy decanoic acid) connected to mono- or di-rhamnose through a glycosidic linkage (Roy, 2017).
2. Trehalolipids - There are several different structural forms of trehalolipid biosurfactants. Trehalose is a non-reducing disaccharide with α,α - 1,1-glycosidic bond connecting the two glucose units. It is the fundamental element of the glycolipids found in the cell walls of *Mycobacteria* and *Corynebacteria*.
3. Sophorolipids - Sophorolipids derived from yeasts are the most efficient glycolipids in terms of surface-active characteristics (White *et al.*, 2013). Sophorolipids are capable of reducing surface and interfacial tension, however, they are ineffective emulsifiers. At least six to nine distinct hydrophobic sophorosides are present in the combination of sophorolipids, which are dimeric carbohydrates sophorose connected to a long-chain hydroxy fatty acid (Hommel *et al.*, 1987).

Lipopeptides

This biosurfactant family comprises cyclic peptides linked to a fatty acid. Lipopeptides can function as particular toxins, enzyme inhibitors, immunomodulators, antifungal, antibacterial, antiviral, and anticancer drugs. One of the most potent and effective biosurfactants is the cyclic lipopeptide surfactin, which is produced by the bacteria *Bacillus subtilis*. Different bacterial strains have different lipopeptide hydrophobicities.

Fatty acids and Phospholipids

It is well-known that phospholipids make up a significant portion of microbial membranes. When growing on n-alkanes, many bacteria and yeasts produce significant quantities of fatty acids and phospholipid molecules. Alkanes undergo microbial oxidations that result in the production of fatty acids, which have been used as surfactants. According to Rahman and Gakpe (2008), microorganisms also create complex fatty acids with OH groups and alkyl branching.

Polymeric surfactants

Emulsan, alasin, liposan, lipomanan, and a few additional lipopolysaccharide and polysaccharide-lipid (or protein) complexes are among the best-studied polymeric biosurfactants (Fenibo *et al.*, 2019). The majority of these biosurfactants are proteins containing polymeric heterosaccharides. According to Lang (2002), emulsan is an emulsifier for hydrocarbons in water at concentrations between 0.001% and 0.01%. Liposan has 83% carbs and 17% protein.

Particulate surfactants

Some bacteria create external membrane vesicles that divide hydrocarbons into microemulsions. They create microemulsions that are crucial for microbial cells to absorb alkane (Desai and Banat, 1997). Vesicles made up of proteins, phospholipids, and lipo-polysaccharides are seen in *Acinetobacter* spp.

MICROORGANISMS PRODUCING BIOSURFACTANTS

A number of microorganisms create biosurfactants, which are mostly released extracellularly or adhered to cell surfaces during growth on water-immiscible substrates. Biosurfactants vary in their chemical composition and nature depending on the microorganism that produces them. The isolation of microorganisms that generate tensioactive compounds with favorable surfactant properties, such as a low CMC, low toxicity, and strong emulsifying activity, has attracted more scientific attention in recent years. A list of microorganisms that generate biosurfactants is shown in Table 1.

Table 1: Biosurfactant-producing Microorganisms

S.NO.	BIOSURFACTANTS	MICROBIAL SOURCE	REFERENCES
1	Rhamnolipids	<i>Pseudomonas aeruginosa</i>	(Jadhav <i>et al.</i> , 2011)
		<i>Pseudomonas chlororaphis</i>	
		<i>Serratia rubidea</i>	
2	Sophorolipids	<i>Candida bombicola</i>	(Casas <i>et al.</i> , 1997)
		<i>Candida batistae</i>	(Konishi <i>et al.</i> , 2008)
		<i>Trichosporon ashii</i>	(Chandran, 2010)
3	Saphorose Lipid	<i>Torulopsis bombicola</i>	(Kim <i>et al.</i> , 1997)
4	Trehalose lipids	<i>Rhodococcus erythropolis</i>	(Muthusamy <i>et al.</i> , 2008)
		<i>Arthrobacter Sp.</i>	
		<i>Nocardia erythropolis</i>	
		<i>Corneobacterium sp.</i>	
5	Ornithine lipids	<i>Pseudomonas sp</i>	(Desai,1997)
		<i>Thiobacillus thiooxidans</i>	
		<i>Agrobacterium sp</i>	
6	Viscosin	<i>Pseudomonas fluorescens</i>	(Banat <i>et al.</i> , 2010)
		<i>Leuconostoc mesenteroids</i>	
7	Carbohydrate lipid	<i>Pseudomonas fluorescens</i>	(Lotfabad <i>et al.</i> , 2009)
		<i>Debaryomyces polymorphus</i>	

8	Cellobiose lipids	<i>Ustilago maydis</i>	(Teichmann <i>et al.</i> , 2007)
9	Aminoacids lipids	<i>Bacillus sp.</i>	(Cotter <i>et al.</i> , 2005)
10	Phospholipids	<i>Acinetobacter sp.</i>	(Kosaric, 2001)

APPLICATION OF BIOSURFACTANTS

Wide ranges of applications can be seen for biosurfactants. Hydrophilic and hydrophobic properties of biosurfactants decrease the surface tension between block hydrogen and immiscible or miscible liquids. In addition to this, due to their substrate specificity, rapid, controlled inactivation and degradation properties, biosurfactants can be used in remediation technology in hydrocarbon and microbial-enhanced oil recovery, food, commercial laundry detergent, paint industries, petrochemicals, medicine, textiles, pollution control as well as mediated biosynthesis of metallic nanoparticles

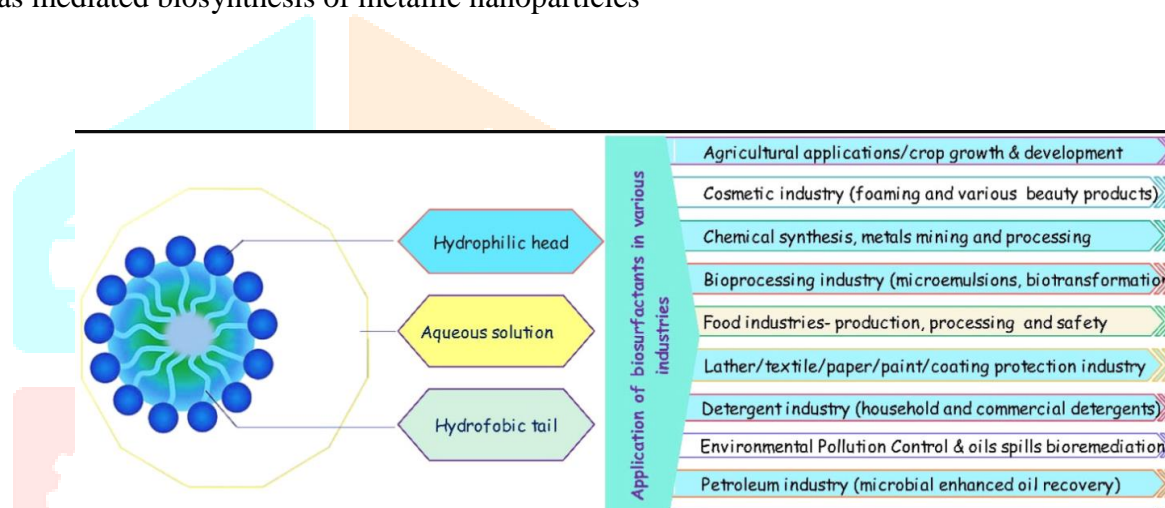


Figure 3: Applications of biosurfactants

Application of biosurfactants in agriculture

Method for Improving Solubility of Biohazardous Chemicals Compounds such as PAHs use surfactants as fluidizers. This increases the apparent solubility of the Hydrophobic Organics Contaminant (HOC). Surfactants are also said to help microorganisms Adsorbs to polluted soil particles and reduce pollution. The diffusion path length between the site of microbial absorption and the site of uptake. Surfactants are also used in agriculture to hydrophilize heavy soils to achieve good wettability. This is to distribute the fertilizer evenly in the soil also it prevents certain fertilizers from hardening during storage and facilitates the spreading and penetration of toxins in pesticides (Makkar and Rockne, 2003). rhamnolipid biosurfactants produced mainly by the genus *Pseudomonas* are known to have strong antibacterial activity. In addition, it does not adversely affect the human body or the environment. Expected based on overall exposure to rhamnolipid biosurfactants. It has also been reported that fengycin has antifungal effects. It can be used for the biological control of plant diseases (Kachholz and Schlingmann, 1987). Biosurfactants are considered an important part of modern agriculture (Mnif and Ghribi, 2015). Hassen *et al.*, (2018) reported that the biosurfactant was derived from *Pseudomonas sp.* And *Burkholderia* species could potentially be used as safe biopesticides. Additionally,

these insecticides can be made from cationic, anionic, anionic, and amphoteric surfactants. Certain biosurfactants of microbial origin have antibacterial effects against plant diseases. They can be considered as promising biocontrol agents for sustainable agriculture. Biosurfactants promote bioregulatory mechanisms of Plant Growth-promoting (PGR) microorganisms such as parasitism, antibiotics, competition, induced systemic resistance, and low toxicity. Pesticides made from biosurfactants can be effectively applied to a wide variety of crops. This has opened the door to stimulating the agrochemical industry to combine different biosurfactant mixtures and polymers to formulate new chemicals for different agricultural applications.

Medical applications of biosurfactants



Figure 4: Applications of biosurfactants in medicine

Antimicrobial Activity: The diverse structure of biosurfactants allows them to function in different ways. Due to their structure, biosurfactants are toxic to the permeability of cell membranes, mimicking detergent-like effects (Zhao *et al.*, 2010). Gharaei-Fathabad (2011) reported that some biosurfactants do. Strong antibacterial, antifungal, and antiviral effects. Besides being therapeutic and probiotic, these surfactants act as anti-adhesion agents against pathogens and are useful in treating many disease-causing agents. A good example is a biosurfactant produced by marine *Bacillus circulans* that exhibits potent antibacterial activity against Gram-positive and Gram-negative pathogens and semi-pathogenic microbial strains, including MDR strains.

Anticancer activity: Some microbial extracellular glycolipids induce cell differentiation rather than cell proliferation in human promyelocytic leukemia cell lines. Moreover, exposure of PC12 cells to her MEL enhanced and inhibited acetylcholinesterase activity. Cell cycle in G1 phase with neurite overgrowth and partial cell differentiation. This suggests that MEL provides the basis for inducing neural differentiation of her PC12 cells and using microbial extracellular glycolipids as novel reagents for this purpose treatment of cancer cells (Krishnaswamy *et al.*, 2008).

Anti-adhesion agents: Biosurfactants have been found to inhibit the adherence of pathogenic microorganisms to solid surfaces or during infection Site, Rodriguez *et al.*, (2006) showed that precoating vinyl urinary catheters by passing them through a surfactin solution prior to medium inoculation reduced the amount of biofilms formed by *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli* and *Mirabilis proteus*. Also pretreatment of silicone rubber with *Salmonella thermophilus* surfactants Inhibited Adherence of *Candida albicans* for 85% .

Immunological Adjuvants: Bacterial lipopeptides are effective non-toxic, non-pyrogenic immunological adjuvants when mixed with conventional antigens increases humoral immune response has been demonstrated with low molecular weight antigens such as iturin AL and herbicolin A .

Gene delivery: Gharaei-Fathabad (2011) stated that the establishment of an efficient and safe method for introducing exogenous nucleotides into mammalian cells is critical for basic sciences and clinical applications.

Biosurfactants as greener alternatives in the food industry

Biosurfactants also show potential applications in the processing industry. It has unique characteristics such as Emulsion, non-adhesion, antimicrobial action for improved food processing. These properties improve the surface emulsification of the product and provide high stability, safe and healthy food for consumer health on a large scale. Nitschke and Silva.(2018) investigated the potential use of biosurfactants in the food industry on a laboratory scale (Giri *et al.*, 2017). They confirm that biosurfactants are safe to use as food additives or can remove heavy metals from products. They also argued that biosurfactants may improve the removal of toxic substances from food crops, vegetables and soil. Studies shows that *Bacillus* sps. MTCC 5877 can remove about 73% of Cd from vegetables, while the biosurfactant produced from *Pseudomonas putida* can remove 50% of Zn compared to the control. Overall, biosurfactants have higher anti-biofilm and anti-biofilm efficacy compared to synthetic surfactants. Antibacterial activity associated with food pathogens. This makes biosurfactants one of the main additives in food processing.

Application of biosurfactants in the cosmetic industry

In the cosmetic industry, biosurfactants have been used as alternatives to synthetic surfactants due to their emulsifying, foaming, water-binding, spreading, and wetting properties that affect viscosity and product consistency. The surfactants are used as emulsifiers, foaming agents, solubilizers, detergents, antibacterial agents, mediators of enzymatic action, insect repellents, antacids, bath additives, acne pads, anti-dandruff products, contact lens solutions, baby products, mascaras, lipsticks, toothpaste, dentin cleaner, etc. Gharaei-Fathabad E (2011).

Application of biosurfactants to bionanotechnology

The integration of microbial-derived biosurfactants and nanoparticles is now considered to be the next generation of alternative green chemistry or bionanotechnology sources. Synthesis of nanoparticles via biosurfactants has great potential for remediation of polluted environments (Christopher *et al.*,2018). However, the synthesized biosurfactant-mediated nanoparticles should be inexpensive. Additionally, it requires no energy consumption, is highly efficient at removing pollutants, and is environmentally sustainable. Studies shows that microorganisms in biosurfactants can stabilize and reduce nanoparticle formation. Similarly, Rane *et al.*, (2017) showed that nanoparticles such as gold and silver can be produced from microorganisms. Generation of this biologically active nanoparticle opens up new visions for other

researchers and scientists Prepare metal nanoparticles using a biosurfactant reducing agent. Kumar *et al.*, (2010) reported that biosurfactants can be produced from *Brevibacterium casei* MSA19. By reducing the number of nanoparticles and suppressing the formation of aggregates due to electrostatic attraction, it is possible to maintain stable nanoparticles for about two months, so they can be used for various product services as an environmentally friendly material. However, research on nanoparticle stabilization by biosurfactants is still in its early stages. Therefore, further research on the stabilization of nanoparticles using biosurfactants is urgently needed before using biosurfactants for various nanotechnology applications.

Application of biosurfactants in commercial laundry detergents

Nearly all surfactants are important components of modern surfactants Commercial detergents are chemically synthesized and is toxic to living freshwater organisms. Increased publicawareness of relevant environmental hazards and risks ofchemical surfactants are stimulating the search for eco-friendly natural substances .Alternative to chemical surfactants found in detergents. biosurfactant Cyclic Lipopeptides (CLPs) and others are stable over a wide pH range (7.0-12.0), heating to high temperatures doesn't cause any variation in surfactant properties (Mukherjee. 2007) . showed good emulsification ability with vegetable oil and shows good Compatibility and stability with commercial detergents advocated for their inclusion in detergent formulations (Das and Mukherje. 2007) .

Application of biosurfactants for oil extraction in petroleum industry

Biosurfactants can also be used in the petroleum industry to clean up oil spills, remove oil residue from storage tanks, and recover oil by microbes. Many researchers have reported that biosurfactants are more selective than chemical surfactants. As such, they are needed in smaller quantities and are more effective for a wider range of oil and reservoir conditions. It can also be used in various industrial processes to enhance solubility, lubricity, mobility, and removal of contaminants and abrasives (Karlapudi *et al.*,2018).

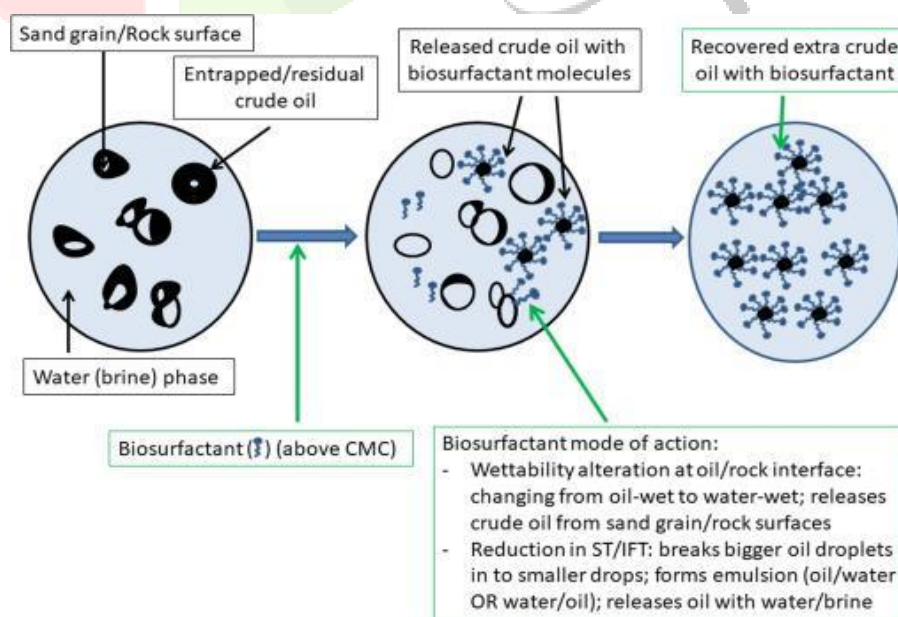


Figure 5: Action of biosurfactants in oil recovery

Nerulkar *et al.*, (2012) conducted a study on a biosurfactant prepared from *Bacillus licheniformis* JF-2 in petroleum-infused water and found that the biosurfactant was more potent and thermostable compared to other surfactants. , was found to be anaerobic. In another study, Das (2018) used biosurfactants prepared from his two strains of *Bacillus subtilis* MTCC1427 and MTCC2423 to recover oil from saturated sand oil columns. The authors showed that the biosurfactant could recover about 62% of the oil in situ used in experiments, it is more stable in the pH range of 4.5-10.5. Biosurfactants also used for biosolubilization, biosorption, and bioremediation of environmental contaminants. They argued that it could also be used in the oil industry for microbial enriched oil recovery, oil immobilization and storage tanks, and oil immobilization due to the stable environmental conditions. Similarly, (McClements and Gumus. 2016) reported that biosurfactants may act as emulsifiers or demulsifiers, removing hydrophobic contaminants from the environment. Furthermore, we report that biosurfactants have higher emulsifying activity compared to synthetic surfactants, improving their ecological compatibility with the environment.

CONCLUSION

Biosurfactants are surface-active molecules produced by microorganisms, either on the cell surface or secreted extracellularly. Biosurfactants have become attractive microbial products in the emerging biotechnology industry due to their advantages over synthetic surfactants in terms of environmental sustainability, global public health, and the concerns of industries to produce environmentally friendly goods.

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