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A REVIEW ON BIOSURFACTANTS AND ITS ENVIRONMENTAL APPLICATIONS

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ABSTRACT

Biosurfactants, derived from biological sources, are widely recognized for their effectiveness as active agents. Among these, glycolipid biosurfactants hold great importance in the field of biotechnology. Various microorganisms, such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida sp*., have been extensively studied for their ability to produce glycolipid biosurfactants. Microbial biosurfactants offer significant advantages over chemical ones, including biodegradability, renewability, and effectiveness even under harsh conditions. Notably, hydrocarbon degrading microorganisms in oil spill areas were found to produce unexpectedly large quantities of biosurfactants due to their lipid metabolism regulation. At present, biosurfactants play a crucial part in the petroleum industry by facilitating the process of emulsification during the recovery and restoration processes at pollution sites. Additionally, they contribute to heavy metal removal in metallurgical industries. This paper provides an overview of the screening of microorganisms that produce biosurfactants, the production methods, and factors influencing biosurfactant production. The review sheds light on the significant role of biosurfactants in environmental cleaning.

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INTRODUCTION

Microorganisms naturally produce biosurfactants, which serve vital roles in the environment. These compounds are categorized into groups such as glycolipid, phospholipid, and lipopeptide. Glycolipid biosurfactants, specifically those that contain sugar molecules and hydroxyl fatty acids, exhibit a combination of hydrophilic and hydrophobic properties that make them valuable as surfactants, emulsifiers, and bioactive compound (Mukherjee *et al.,* 2006). Compared to synthetic alternatives, biological surfactants offer advantages such as high biodegradability, non-toxicity, renewability, and rapid production. Furthermore, they exhibit favorable attributes such as effective detergent action, foaming ability, wetting properties, and the capacity to create micro-emulsions. Biosurfactants are also used in harsh environments with high pH, salinity, and temperature (Desai *et al.,* 1997). *Pseudomonas aeruginosa* stands out as a microorganism with remarkable biosurfactant production abilities, as it can degrade a wide range of substrates. Furthermore, inexpensive raw materials such as discarded oil, soap residue, and other by products originating from food sectors and vegetable oil refineries are commonly used for biosurfactant production, with vegetable-based oils yielding high biosurfactant quantities (Jarvis *et al.,* 1949). Glycolipid biosurfactants have been identified as particularly promising due to their environmental remediation capabilities, non-toxic nature, and biodegradability. These biosurfactants find extensive applications in diverse sectors, including cleansing agents, pharmaceuticals, therapeutics, cosmetics, heavy metal removal, agriculture, and oil recovery (Mulligan *et al.,* 2004) Overall, the properties of biosurfactants exhibit similarities, but glycolipid biosurfactants offer distinct advantages, making them highly desirable for various applications.

Classification of Biosurfactants: Typically, surfactants that are created through chemical synthesis are categorized according to the characteristics of their polar constituents. The categorization is primarily determined by the specific chemical composition, which results from various molecules forming both the hydrophobic and hydrophilic portions, and whether they originate from microbial sources. The hydrophobic portion consists of saturated or unsaturated fatty acidsand the hydrophilic segment can encompass carbohydrates, cyclic peptides, amino acids, phosphate groups, carboxylic acids, or alcohols (Desai *et al.*, 1997). A classification for biosurfactants was proposed, dividing them into two groups: low-molecular-weight compounds primarily responsible for reducing surface and interfacial tension, and high-molecular-weight polymers that effectively function as stabilizers for emulsions (Rosenberg *et al.,* 1999). Notable examples of surfactants with low-molecular mass comprise lipopeptide, lipopeptides, and phospholipids. On the other hand, high-molecular-weight biosurfactants encompass particulate and polymeric

surfactants, such as polyanionic hetero-polysaccharides that contain both polysaccharides and proteins. The production of surfactants secreted by microbes is influenced by the nutritional environment of the microorganism during growth. For reference, a list of the most significant categories of biosurfactants is provided (Table 1).

Procedure of Hydrocarbon Utilization: Although the bacterial uptake of alkanes are widely considered as passive transport, microorganisms possess various adaptive mechanisms to accumulate and transport hydrocarbons inside the cell for initial enzymatic breakdown (Hommel *et al.,* 1990). They have the ability to move and integrate soluble alkanes present in the aqueous phase, and it was previously believed that only dissolved hydrocarbons could be utilized by bacteria (Britton *et al.,* 1984). However, the degradation rates of alkanes exceed the dissolution rates in the aqueous phase, indicating the use of other uptake mechanisms by hydrocarbon-degrading microorganisms (Leahy*et al.,* 1990). Different theories have been proposed to explain the uptake of aliphatic hydrocarbons, ruling out the possibility of long-chain alkanes being transported through the water phase in a dissolved state (Singer et al., 1984). During the uptake of hydrocarbons, small droplets of hydrocarbon are encapsulated within the cells (referred to as micelles), and direct cell contact with the larger hydrocarbon phase enables the cells to take up hydrocarbons. It is noted that hydrocarbon-degrading microorganisms adapted to oil-consisting surroundings play a crucial part in biologically treating pollution (Ron et al, 2002). One limiting factor, particularly at low temperatures, is the bioavailability of various oil fractions. To address this, microorganisms capable of breaking down hydrocarbons create biosurfactants with varying chemical compositions and molecular sizes.

Here are some of the screening methods used for biosurfactant-producing microorganisms:

1. ***Hydrocarbon Overlay Agar Test:*** This method involves observing colonies on agar plates coated with oiland encircled by emulsified halo zones, indicating utilization of hydrocarbons through biosurfactant production and potential biosurfactant producers (Morikawa *et al.,* 1992)
2. **CTAB Agar Plate:** Suitable for categorizing rhamnolipids, this method results in a dark blue halo zone around colonies due to the formation of insoluble ion pairs between the anionic biosurfactant and cationic CTAB-MB present in the medium (Siegmund *et al.,* 1991, Pradhan AK et al., 2013).
3. **Haemolytic Activity:** This method identifies the presence of biosurfactants by observing the rupture of red blood cells, but it is considered an unreliable criterion for biosurfactant activity detection (Banat *et al.,* 1993, Pradhan AK et al., 2024).
4. **Drop Collapse Method:** A simple and widely used screening method where the presence of biosurfactants is noted by the collapse of the hydrocarbon source (Pennzoil) (Bodour *et al.,* 1998).

Emulsification activity is a critical parameter for evaluating biosurfactant-producing microorganisms. One approach is through optical density (Rosenberg *et al.,* 1979), where culture broth's optical density, which contains hydrocarbon is in contrast to that of culture broth alone, revealing it's emulsification activity. Another approach is the emulsification index, which calculates the emulsion layer formed between the aqueous and kerosene layers to determine the emulsification activity and stability, providing insights into the strength of the biosurfactant (Cooper *et al.,* 1987, Ellaiah *et al.,* 2002).

Table 1. Varieties of biosurfactants and their producing microorganisms

Different types of microbial surfactants	Organisms associated with their production
Glycolipids	*Alcanivorax borkumensis* *Serratia marcescens,* *Corynebacterium sp.,* *Arthrobacter sp.*
Rhamnolipids	*Pseudomonas sp.,* *Serratia rubidea,* *Pseudomonas aeruginosa*
Sophorolipds	*Torulopsis apicola,* *T. bombicola* *T. petrophilium,* *Candida lipolytica,* *Candida apicola,* *Candida bombicola,* *Candida bogoriensis.*
Trehalose lipids	*Rhodococcuserythropolis,* *Nocardiaerythropolis,* *Mycobacterium sp.,* *Arthrobacter paraffineus,* *Corynebacterium sp*
Fatty Acids (Spiculisporic Acids, Corynomycolic Acids, etc.,)	*Candida lepus,* *Capnocytophaga sp.,* *Corynebacterium lepus,* *Penicillium spiculisporum,* *Norcadia erythropolis*
Carbohydrate-lipid-protein	*Pseudomonas fluorescens*
Mannan-lipid-protein	*Candida tropicalis*
Particulate Surfactants	*Pseudomonas marginalis*

Screening of Microorganism: The initial stage in the selection process involves isolating strains from their natural habitats. After isolation, the screening of specific microorganisms to identify those producing the desired product becomes crucial in the biological processing of microbial cultures. Primary screening involves various highly selective techniques to detect and isolate microorganisms that produce the desired metabolite. Ideally, primary screening should be swift, cost-efficient, predictive, specific, and capable of scalability. Nonetheless, it can be a time-consuming and labor-intensive process because it necessitates the screening of a considerable number of isolates to identify potential candidates.

Biosurfactant Production: Numerous researchers utilized different bacterial strains to produce biosurfactants through culture media. The majority of these bacteria were isolated from polluted locations, commonly harboring residues of petroleum hydrocarbons and industrial byproducts (Benincasa *et al.,* 2007).

Fermentation Approaches for Biosurfactant Production: Various fermentation techniques are employed in the production of

biosurfactants. Rhamnolipid production, in particular, utilizes various strategies, including batch cultivation, shake flask, continuous, fed-batch and integrated microbial/enzymatic approaches. Researchers also apply genetic manipulation and immobilized culture cultivation methods to increase production of surfactin. Rhamnolipid is classified as a secondary metabolite that is typically produced under specific conditions, often in the presence of growth-limiting substrates, especially carbon sources. Notably, nitrogen and phosphorus are crucially limited compounds in the production of rhamnolipid. Interestingly, nitrate has been found to elevate biosurfactant yield. The key carbon sources used for production of rhamnolipid encompass glycerol, glucose, ethanol, n-alkanes and glycerolipids (Lee *et al.,* 2004). For batch cultivation, biosurfactant generation relies on growth-constraining substances like plant oil or glucose, while glycerol or plant oil assume this role in fed-batch culture. Glucose and hydrocarbon are used as substrates in continuous cultivation. An instance is seen, where he explored solid-state cultivation within continuous fermentation (Camilios *et al.,* 2011). Surfactin production was investigated using glucose as a precursor, and foam fractionation method to separate the product from the reactor (Cooper *et al.,* 1981). Scientists also inspected the production of surfactin using the microbe, *Bacillus subtilis*, by employing a chemostat with stirred tank bioreactor (Noah *et al.,* 2005). Another study utilized an airlift fermentor with continuous foam collection for surfactin production from *Bacillus subtilis*. Potato process effluent was used as the carbon source for this situation. Subsequently, a creative bioreactor design was introduced with specific goal of averting foam overflow during the biosurfactant synthesis process (Yeh *et al.,* 2006). They illustrated the utilization of a bubbleless bioreactor incorporating a hollow fiber membrane to serve as an air-liquid interface for the production of surfactin and fengycin *Bacillus subtilis* (Coutte *et al.,* 2010).

Factors Affecting the Biosurfactant Production: In the process of production of biosurfactant, the production yield is influenced by various factors which are outlined in Table 2.

Effect of Carbon Sources: Microorganisms involved in biosurfactant production utilize various carbon sources and energy for growth. For example, *Pseudomonas aeruginosa* can use ethanol, glucose, glycerol, and mannitol to produce rhamnolipids (Robert *et al.,* 1989). Interestingly, glycerol behaves uniquely, with rhamnolipid production sharply decreasing when its concentration exceeds 2%. Safi et al. found that 3% glycerol yielded only 2 g/L of rhamnolipids (Safi*et al.,* 2007). Similarly, grape seed oil and sunflower oil at 6% concentration produced 2 g/L of rhamnolipids. Glucose at a 6% concentration resulted in a rhamnolipid yield ranging from 1400 mg/L to 1500 mg/L. Diesel and kerosene oil at 6% and 5% concentrations produced 1.3 g/L and 2.1 g/L of rhamnolipids, respectively (Desai *et al.,* 1997). Soybean lecithin and crude oil were found to be suitable carbon sources for biosurfactant production. A study showed that soybean lecithin was slightly more effective than crude oil (Changjun Zoua *et al.,* 2014). However, crude oil proved efficient as a carbon source for Acineto bacter-related bacteria (Huy *et al.,* 1999). In other studies, hydrocarbons like n-hexadecane and paraffin were considered as carbon sources, but water-soluble carbon sources were more readily used for biosurfactant production compared to paraffin and n-hexadecane (Jorge*et al.,* 2013). Nevertheless, it was proposed that a 2% glucose concentration had excellent potential as a carbon source, resulting in a yield of 5.28 g/L of rhamnolipids (Onwosi *et al.,* 2012).

Effect of Nitrogen Source: Nitrogen is considered as a very important source in promoting biomass growth and facilitating production of biosurfactant. *Pseudomonas aeruginosa* has been identified as a favorable strain for biosurfactant production. But, when the nitrogen source becomes scarce, it enters the stationary phase, leading to a decline in biosurfactant production (Ramana *et al.,* 1989). Conversely, an excess of nitrogen source hinders biosurfactant-producing microorganisms, resulting in reduced biosurfactant production (Syldatk *et al.,* 1985). Various nitrate salts, including ammonium nitrate, potassium nitrate, and sodium nitrate, have been investigated to be potential nitrogen sources for synthesis of biosurfactants. Among these, sodium nitrate has been identified to be very effective as a nitrogen source, yielding 4.38 g/l of biosurfactant (Onwosi*et al.,* 2012). In some studies, it was mentioned that ammonium nitrate was identified as the most suitable nitrogen source for production of biosurfactant (Joshi *et al.,* 2012). Additionally, some surveys also demonstrated that potassium nitrate exhibited superior results in contrast to alternative nitrogen sources, like ammonium sulfate or urea, when used for biosurfactant production by *Rhodotorula glutinis IIP-30* (Johnson *et al.,* 1992). Researchers also explored meat and yeast extract as alternative nitrogen sources, which effectively influenced biosurfactant production (Jorge *et al.,* 2013).

Effect of Temperature: The temperature plays a significant role in the production of biosurfactants. Rhamnolipid production showed an increased production between 25°C and 30°C, remained stable between 30°C and 37°C, and slightly decreased at 42°C. Scientists studied how temperature affected the growth of *Pseudomonas aeruginosa* and rhamnolipid synthesis (Vollbrecht *et al.,*1998). In elevated temperatures, like 47°C, the culture growth suffered, resulting in reduced production of rhamnolipid. Similarly, the culture of *Tsukamurella sp*. Faces issues with higher temperatures resulting in cell aggregation which led to reduction in glycolipid production. However, some microbes, like *Acinetobacter baylyi ZJ2*, demonstrated resilience to temperatures ranging from 40–45°C (Changjun Zoua *et al.,* 2014). 30°C was proposed as the ideal temperature, as it facilitated cell growth and elevated glycolipid production. The peak production of biosurfactant occurs at 30°Cby *Pseudomonas aeruginosa* PBSCI, as documented in some studies (Joice *et al.,* 2014).

Table 2. Effects of various factors on biosurfactant production

Sl. No	Microorganisms	Biosurfactants	pH	Temp.	Carbon source	Yield	References
1	*Bacillus brevis*	Lipopetide	8	33°C	8.5g/l of glucose	-	Mouafi *et al.,* 2016
2	*Pleurotus djamor*	Lipopeptide	5.5	29°C	5g/l of sunflower seed shell	8.9±0.5 g/l	Velioglu *et al.,* 2015
3	*Pseudomonas aeruginosa KVD-HR42*	Rhamnolipids	7.8	37°C	23.85g/l Karanja oil	5.90±2.1 g/l	Deepika *et al.,* 2016
4	*Bacillus subtilis ICA 56*	-	8		Glycerol and sunflower oil	1.29 g/l	De Franc *et al.,* 2015
5	*Pseudomonas aeruginosa F23*	Rhamnolipids	8	30°C	1% coconut oil	2.8 g/l	Patil *et al.,* 2014

Effect of pH: The production of biosurfactants is influenced by pH, with a pH range of 6.0-6.5 being found to be optimal (Gobbert *et al.,*1984). Beyond a pH of 6.5, biosurfactant production decreases, and at extremely acidic levels (pH 4 - 4.5), the organism loses its ability to reduce the surface tension of the culture medium, leading to a decrease in biosurfactant yield. Studies have shown that the growth of microorganisms for biosurfactant production is not hampered when the pH is increased from 6.5 to 7.0 (Cooper *et al.,* 1987). On the contrary, reducing the pH negatively impacts surfactant production (Guerra-Santos *et al.,* 1986). Likewise, an alkaline environment with a pH above 7 has been found to retard growth, as demonstrated in a research on *Acinetobacter baylyi* ZJ2 (Changjun Zoua *et al.,* 2014). pH was also observed to affect the metabolism of microorganisms (Joice *et al.,*2014). The pH was adjusted between 5.0 and 8.5 and found that surface tension decreased by 29.19 mN/m at pH 6.5, and emulsification activity reached 75.12% at pH 7.0. In the end, it was determined that *Pseudomonas aeruginosa* PBSC1 produced the most amount of biosurfactant at pH 7.0.

Effect of Aeration and Agitation: Foam accumulation is associated with aeration, and agitation impacts the transport of oxygen and

medium components (Shaligram *et al.,* 2010). Consequently, both agitation and aeration play crucial roles in growth of cells and biosurfactant synthesis, particularly for aerobic microbes. In an observation, the rate of air flow was optimized to 0.75 vvm using the response surface method to enhance biosurfactant production (Sen *et al.,* 1997).In another study, they looked at agitation's impact and observed that increasing the agitation rate from 50 to 200 ppm boosted the growth rate from 0.2 to 0.72 per hour, ultimately reaching an 80% maximum biosurfactant output (Sen *et al.,* 1997). This higher agitation rate also substantially increased the dissolved oxygen level in the system, going from 0.1 to 0.55 mg/l. Consequently, elevated levels of dissolved oxygen significantly enhanced cell growth, leading to greater biosurfactant production (Wei *et al.,* 2005).

metallurgical industries, releasing heavy metals. These toxic substances contaminate soil, water, and infiltrate the food chain, causing severe environmental problems. Recently, methods like excavation have been proposed to remediating metal-contaminated soil and relocating it to designated sites (Asci *et al.,* 2010). In the bioreduction of heavy metals, microorganisms can serve as effective biocatalysts to transform the metals into different forms (Bruins *et al.,* 2000). Bioremediation methods, such as soil flushing and soil washing, are commonly used techniques using biosurfactants to remediate soil contaminated with heavy metal soil. The biosurfactant can be introduced into the soil either in-situ through trenches and drain pipes or the soil is collected and washed in a separate location with a biosurfactant solution, in ex situ process (Singh *et al.,* 2004).

Table 3. Type of biosurfactants, bacteria, solvent and analytical methods Involved

Biosurfactant & Bacteria	Analytical Method	Chemicals/Solvents required	References
Rhamnolipids	HPLC	CH_3CN-H_2O	Schenk *et al.,* 1995
	TLC	$CHCl_3/CH_3OH/CH_3COOH$	Arino *et al.,* 1996
	TLC	CH_3OH/H_2O	Rahman *et al.,* 1999
Pseudomonas aeruginosa	TLC	CH_3CN/H_2O	Caldini *et al.,* 1995
	HPLC	CH_3CN (Contain 2-bromoacetophenone and triethylamine)	Venkatesh *et al.,* 2012
Lipopeptide *Acinetobacter baylyi* ZJ2	FTIR	$CHCl_3/CH_3OH/CH_3COOH$	Zou *et al.,* 2014
Sophorolipid *Candida bombicola*	HPLC with ELSD	CH_3CN/H_2O	Davila *et al.,* 1997
Phospholipid *Acinetobacter sp.*	GC-MS	$CHCl_3/CH_3OH$	Koma *et al.,* 2001
Trehalose lipid *Rhodococcus sp. P32C1*	HPLC	CH_3CN	Maghsoudi *et al.,* 2001
Surfactin *Bacillus Subtilis ATCC 21332*	HPLC	CH_3CN/TFA	Davis *et al.,* 2001

Purification Methods for Biosurfactants: In traditional approaches, the extraction of crude biosurfactants from microbial biomass involved the use of concentrated hydrochloric acid. However, contemporary methods now offer a range of methods for isolation and purification of crude biosurfactants, including membrane-based processes, foam fractionation, absorption, and extraction (Sen *et al.,* 1997). Sen and Swaminathan were among the first to report on membrane separation for surfactin recovery, and they also successfully developed a bubbleless membrane bioreactor which proved to be very effective for biosurfactant production. This innovative bioreactor couples microfiltration and ultrafiltration to enhance the process of separation efficiently (Coutte *et al.,* 2013). Foam fractionation, an effective method for separating biosurfactants, involves the addition of acidified hydrochloric acid to precipitate the biosurfactant, followed by solvent-based collection (Cooper *et al.,* 1981). A study by Davis and team showcased foam fractionation as an integrated system for surfactin isolation (Davis *et al.,* 2001). Extraction techniques have gained considerable attention from researchers due to their ease of operation. Different solvents like methanol, chloroform, ethyl acetate, butanol, dichloromethane, hexane, diethyl ether, pentane, acetic acid and isopropanol are employed to extract biosurfactant. These solvents effectively dissolve hydrophobic moieties, facilitating the extraction of the crude product (Desai *et al.,* 1997). To purify biosurfactants, amberlite XAD 2 or polystyrene resins are used for adsorption and desorption. Factors affecting recovery include agitation rate, carbon particle size, temperature, pH, adsorbent amount, initial concentration, and ionic strength. Polymer resins and organic solvents are advanced techniques, while activated carbon aids in surfactin recovery, and regenerated carbon can also be used for biosurfactant recovery (Liu *et al.,* 2007; Dubey *et al.,* 2005).

ANALYTICAL METHODS

Many researchers have employed various analytical techniques to analyze and characterize biosurfactants. Table-3 shows the different types of biosurfactants, microorganisms, solvent and type of analytical method used.

Environmental application of biosurfactants

Biosurfactants in Metallurgical Industry: In modern times, the environment faces a significant challenge of pollution due to rapid industrialization. One such type of harmful pollution stems from

In the bioreduction of heavy metals, microorganisms can serve as effective biocatalysts to transform the metals into different forms (Bruins *et al.,* 2000). Bioremediation methods, such as soil flushing and soil washing, are commonly used techniques using biosurfactants to remediate soil contaminated with heavy metal soil. The biosurfactant can be introduced into the soil either in-situ through trenches and drain pipes or the soil is collected and washed in a separate location with a biosurfactant solution, in ex situ process (Singh *et al.,* 2004). In the bioreduction of heavy metals, microorganisms can serve as effective biocatalysts to transform the metals into different forms (Bruins *et al.,* 2000). Bioremediation methods, such as soil flushing and soil washing, are commonly used techniques using biosurfactants to remediate soil contaminated with heavy metal soil. The biosurfactant can be introduced into the soil either in-situ through trenches and drain pipes or the soil is collected and washed in a separate location with a biosurfactant solution, in ex situ process (Singh *et al.,* 2004). In the bioreduction of heavy metals, microorganisms can serve as effective biocatalysts to transform the metals into different forms (Bruins *et al.,* 2000). Bioremediation methods, such as soil flushing and soil washing, are commonly used techniques using biosurfactants to remediate soil contaminated with heavy metal soil. The biosurfactant can be introduced into the soil either in-situ through trenches and drain pipes or the soil is collected and washed in a separate location with a biosurfactant solution, in ex situ process (Singh *et al.,* 2004). In the bioreduction of heavy metals, microorganisms can serve as effective biocatalysts to transform the metals into different forms (Bruins *et al.,* 2000). Bioremediation methods, such as soil flushing and soil washing, are commonly used techniques using biosurfactants to remediate soil contaminated with heavy metal soil. The biosurfactant can be introduced into the soil either in-situ through trenches and drain pipes or the soil is collected and washed in a separate location with a biosurfactant solution, in ex situ process (Singh *et al.,* 2004). In another study, removal of Cr(III) from chromium-contaminated kaolinite and found that elevated pH levels and the addition of NaOH positively influenced metal removal (Massara *et al.* 2007).This was attributed to the enhanced chelating effect of biosurfactants at elevated pH levels, resulting in improved metal removal (De Franc *et al.,* 2015). Biosurfactant solubility increased with the addition of NaOH, thereby promoting enhanced metal removal. In another study, removal of Cr(III) from chromium-contaminated kaolinite and found that elevated pH levels and the addition of NaOH positively influenced metal removal (Massara *et al.* 2007). This was attributed to the enhanced chelating effect of biosurfactants at elevated pH levels, resulting in improved metal

Table 4. Removal of Heavy Metals by Biosurfactant Producing Organism

S.no	Metals	Microorganism	Removal (%)	Reference
1.	Cr	*Pseudomonas aeruginosa*	46	Hassen *et al.,* 1998
		Aspergillus niger	21-36	Dursun *et al.,* 2003
2.	Cd	*Bacillus strain* H9	36	Roane *et al., 2001*
		Aspergillus terreus	70	Massaccesi *et al., 2002*
		Pseudomonas aeruginosa	73.2	Wang *et al.,* 2004
3.	Cu	*Thiobacillus ferrooxidans*	25	Boyer *et al.,* 1998
		Schizosaccharomyces pombe	11-25	Donmez *et al.,* 1999
4.	Pb	*Pseudomonas aeruginosa* PU21	80	Chang *et al.,* 1997
		Aspergillus niger	13-88	Dursun *et al.,* 2003
5.	Ni	*Pseudomonas spp.*	98	Magyarosy *et al., 2002*
		Candida spp	29-57	Donmez *et al.,* 2001
		Pseudomonas aeruginosa	68.1	Wang *et al.,* 2004

Table 5. Recovery of oil by using various biosurfactants

S. No	Biosurfactants Producing Organisms	Biosurfactants	Biosurfactant yield	Recovery of Oil from Oil Contaminated Soil (%)	References
1.	*Bacillus subtilis* CN2	Lipopeptide	7150mg/l	84.6 ± 7.1	Bezza *et al.,* 2015
2.	*Bacillus subtilis* BS-37	Surfactin isoform	585mg/l	96	Liu *et al.,* 2015
3.	*Bacillus* strain		Crude BS 0.081- 1 g/l CMC Value19.439mg/l	30.22 – 34.19	Joshi *et al.,* 2013
4.	*Bacillus subtilis* B 30	Surfactin	Crude BS 0.3 – 0.5 g/l CMC Value 1:8	17-26	Al-Wahaibi *et al.,* 2014
5.	*Candida sphaerica*	Anionic biosurfactants	4.5g/l	75 (Clay soil) 92 (Silty Soil)	Sobrinho *et al.,* 2008
6.	*Candida tropicalis*		3.61± 2.1	78 - 97	Batista *et al.,* 2010
7.	*Candida glabrata* UCP 1002		7.52g/l	92.6	Gusmao *et al.,* 2010
8.	*Candida sphaerica*UCP 0995	Biosurfactant Lunasan	9g/l	95	Luna *et al.,* 2013

removal (De Franc *et al.,* 2015). Biosurfactant solubility increased with the addition of NaOH, thereby promoting enhanced metal removal.

Biosurfactants in petroleum industry: Microorganisms that produce biosurfactants, whether indigenous or introduced, are employed to enhance recovery of oil in wells that produce them. This involves the directly injecting the nutrients along with certain microorganisms which have the ability to produce desired products to mobilize oil or to implement microbial-enhanced oil recovery. This method includes reducing surface tension/oil viscosity and repressurizing the reservoir. By injecting biosurfactants, some specific bacterial species such as *Bacillus licheniformis* and *Pseudomonas aeruginosa*, along with the nutrients, have demonstrated the ability to increase the recovery of oil by 30-200% (Singh *et al.,* 2008). This approach is particularly efficient for extracting oil from high-viscosity crude oil or reservoirs with low permeability. The petroleum industry faces significant challenges with oil field emulsions occurring at various stages during crude oil processing. To address this, the de-emulsification process, involving centrifugation, heat treatment, and chemicals, has proven effective. However, biosurfactants offer an eco-friendly alternative by replacing chemical de-emulsifiers in situ. Certain bacterial species, including *Acinetobacter* and *Pseudomonas*, act as key de-emulsifiers in mixed cultures (Nadarajah *et al.,* 2002). These microorganisms employ a range of biosurfactants, including phospholipids, polysaccharides, glycolipids, and glycoproteins, to disrupt emulsions by harnessing the amphiphilic properties of these compounds or the hydrophobic characteristics of their cell surfaces, displacing emulsifiers from the oil-water interface (Mukherjee *et al.,* 2006). Biosurfactants exhibit the capacity to recover oil from petroleum tank bottom sludges and enhance the transportation of heavy crude in pipelines. Rhamnolipids have shown effectiveness in removing soaked oil from used oil sorbents, achieving up to 95% oil removal, while the application of fermentation broth has efficiently removed crude oil from contaminated sites (85%) and motor oil (90%) (De Franc *et al.,* 2015). The rates of oil recovery by using various biosurfactants are given in Table-5.

CONCLUSION

The objective of this review article is to present a concise and reader-friendly understanding of the diverse perspectives surrounding biosurfactants. Emphasizing their significance for environmental applications, the paper highlights the potential of biosurfactants in promoting eco-friendly natural processes and accelerating production rates. Extensive research has resulted in the identification of multiple strains suitable for large-scale biosurfactant manufacturing, and the paper outlines screening methods for identifying these producers. Additionally, this paper explores different operational factors that influence the production process. In order to maintain product purity, this paper provides a concise overview of analytical methods employed for biosurfactant purification, including HPLC, TLC, GC-MS, foam fractionation, and membrane separation techniques. Furthermore, the application section delves into the role of biosurfactants in industries associated with oil and metal. Overall, this comprehensive review simplifies the subject matter, making it accessible for readers.

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