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A Review on Biosurfactants and its Environmental Applications

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Abstract: *Biosurfactants are widely known as surface active agents of biological origin. Glycolipid classes of biosurfactants has high important in the biotechnological arena. Pseudomonas aeruginosa, Bacillus subtilis, and Candida sp., are important classes of microorganism and highly investigated for the production of glycolipid biosurfactants. Commercially, microbial biosurfactants are more advantageous than chemical based biosurfactants due to their biodegradability, renewability and functionality maintenance under extreme operating conditions. In the oil spill areas hydrocarbon degrading microorganisms (isolated) were proven to produce enormous amount of biosurfactant than expected. This is due to the regulation of all the genomes in the synthesis of lipid metabolism. Currently, biosurfactants play vital role in oil and petroleum industries for emulsification in both recovery and removal process from the site of pollution. In addition, it has some role in the heavy metal removal of metallurgical industries. In the present paper, we have given the overview on screening of biosurfactant producing microorganisms, production methods and factors influencing the production of biosurfactants are investigated. Various analytical techniques used for processing of crude metabolites are also presented. Thus from this review, can easily understood the role of biosurfactant in the environmental cleaning.*

Keywords: *Biosurfactants, Emulsification, Environmental cleaning, Hydrocarbon*

I. Introduction

Biosurfactants are surface active compounds produced by microorganism which have indispensible environmental applications. There are different groups of biosurfactants in the form of glycolipid, phospolipid and lipopeptide. The glycolipid biosurfactants containing sugar molecule and hydroxyl fatty acids were found to have hydrophilic and hydrophobic activities. The later type was reported to have some functional roles such as surfactant, emulsifier and bioactive. Generally, biological surfactants are highly biodegradable, non-toxic and renewable and may perform over synthetic surfactants with high surface tension, interfacial tension and critical micelle concentration. These can be produced easily in a short interval of time. Biological surfactants have excellent detergent, foaming, wetting, and micro-emulsifying properties[1]. It can be operated at high pH, salinity and temperature[2]. Generally, a surfactant can reduce the surface tension of water to 25-40 mN/m and having critical micelle concentration value of 5-380 mg/l. Similarly interfacial tension decreases in oil and water to < 1 dyne/cm. *Pseudomonas aeruginosa* is a special microorganism for the production of biosurfactants because of its ability to degrade wide variety of substrates. Cheap raw materials used for the biosurfactant production are oil waste, soap stock and other waste from food industries and vegetable oil refineries. Amongst the entire carbon sources, vegetable based oil is found to have higher biosurfactant yield[3].

Moreover, the properties of biosourfactants are similar to each other. However, glycolipid biosurfactants were reported as promising biosurfactants with various advantages. Some of these advantages are environmental remediations, non-toxic and biodegradable. There are wide applications of biosurfactants in various sectors such as pharmaceuticals, therapeutics, cosmetics, detergents, agriculture and removal of heavy metals and in oil recovery[4].

II. General Classification of Biosurfactants

Chemically synthesized surfactants are usually classified depending on the nature of their polar groups. Normally they are categorized mainly by their chemical composition obtained by the different molecules forming the hydrophobic and hydrophilic moieties and microbial origin. The compositions of hydrophilic moiety are amino acids, peptides, mono, di, or polysaccharides and that of hydrophobic moiety are saturated or unsaturated fatty acids[2]. Rosenberg and Ron[5] suggested that biosurfactants can be classified into a low-molecular-mass molecules, which mainly lower surface and interfacial tension, and high molecular-mass polymers, which are effective emulsion stabilizing agents. The major classes of low mass surfactants include glycolipids, lipopeptides and phospholipids, whereas high mass surfactants include polymeric and particulate surfactants like polyanionic hetero-polysaccharides containing both polysaccharides and proteins. The microbial

surfactant production varies according to the nutritional environment of the growing microorganism. The most important types of biosurfactants are listed in Table 1.

Table 1: Type of Biosurfactants and Micro-organism Involved

Types of Microbial Surfactants	**Organisms involved**
Glycolipids	*Serratia marcescens, Alcanivorax borkumensis, Arthrobacter sp., corynebacterium sp.,*
Rhamnolipids	*Pseudomonas sp., Pseudomonas aeruginosa, Serratia rubidea*
Sophorolipids	*Torulopsis apicola, T. bombicola* *T. petrophilium, Candida apicola, Candida bombicola, Candida bogoriensis, Candida lipolytica*
Trehalose lipids	*Rhodococcus erythropolis, Nocardia erythropolis, 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*

III. Mechanism of Hydrocarbon Utilization

Although the actual uptake of alkanes by bacteria is thought to be a passive transport process, microorganism possesses a number of adaptive mechanisms for accumulating and transporting hydrocarbons into the cell for initial enzymatic catabolism[6]. Bacteria can transport and assimilate soluble alkanes that are dissolved in the aqueous phase. Indeed, it was initially thought that bacteria could utilize only solubilised hydrocarbon[7]. However, alkanes are degraded at rates which exceed the rates of dissolution of hydrocarbon in the aqueous phase, indicating that other uptake mechanism are also utilized by hydrocarbon degrading microorganism[8]. Different mechanisms for the uptake of aliphatic hydrocarbons have been proposed. Due to the low solubility of long chain alkanes, a transport through the water phase in a dissolved state was ruled out[9].The following steps are found to occur during the uptake of hydrocarbon a) small hydrocarbon droplets (micelles) are enclosed into the cells. b) The direct contact of cells to the bigger hydrocarbon phase enables the cells to enclose hydrocarbon into their cells.

Ron and Rosenberg[10] reported that hydrocarbon degrading microorganism adapted to grow in oil containing environment have an important role in the biological treatment of the pollution. One of the limiting factors in this process especially at low temperature is the bioavailability of many fractions of the oil. The hydrocarbon degrading microorganisms produce biosurfactants of diverse chemical nature and molecular size.

IV. Screening of Microorganism

Isolation of the strains from their natural habitats is the initial step in the selection stage. Followed by isolation, screening of specific microorganism for the production of desired product plays a significant role in the bio-processing of microbial cultures. A set of highly selective procedures, which allows the detection and isolation of microorganisms producing the desired metabolite, constitutes primary screening. Ideally, primary screening should be rapid, inexpensive, predictive, specific but effective for a broad range of compounds and applicable on a large scale. Primary screening is time-consuming and labour intensive since a large number of isolates have to be screened to identify a few potential ones. The various screening methods adopted for bio-processing of biosurfactant producing microorganism are briefly discussed below.

Hydrocarbon overlay agar test is one of the efficient method in which the colonies formed in oil coated agar plates are surrounded by emulsified halo zones. The zone indicates that the microorganisms in the colonies utilizes hydrocarbons through biosurfactant production and hence a potential biosurfactant producers[11]. If the biosurfactant produced is categorized as rhamnolipids, then CTAB agar plate is the suitable screening method. In this method, the anionic biosurfactant forms insoluble ion pair with cationic CTAB-MB present in the medium and hence a dark blue halo zone is produced around the colonies[12]. Haemolytic activity is another screening method in which the rupture of red blood cells is identified in the presence of biosurfactants. However this method is an unreliable criterion for the detection of biosurfactant activity[13].A simple method called drop collapse method is also widely used for screening biosurfactant producing organisms. The collapse in the pennzoil (hydrocarbon source) is noted for the presence of biosurfactants[14]. If the biosurfactant concentration is very low, this method gives negative results[15].

Emulsification activity is one of an important parameter to evaluate biosurfactant producing microorganisms. The first approach of emulsification activity through optical density is developed by Rosenberg *et al*[16] and later modified by Neu and Poralla[17.] Here, the optical density of culture broth added with hydrocarbon is compared with that of culture broth alone and the difference of which yields the emulsification activity. Another approach of emulsification activity is achieved through emulsification index. An emulsion layer formed between aqueous and kerosene layer is calculated and utilized for emulsification index. Emulsification index stability gives the knowledge on the strength of biosurfactant[18, 19].

V. Biosurfactant Production

Many researchers employed various kinds of bacteria in producing biosurfactant using culture media. Most of the bacteria used are isolated from contaminated sites usually containing petroleum hydrocarbon byproducts and industrial wastes[20].

5.1 Fermentation Strategies for Biosurfactant Production

Various fermentation strategies are adopted for the production of biosurfactant. In general the following are used in the rhamnolipid production such as shake flask, batch, fed-batch, continuous and integrated microbial/enzymatic process. In addition genetic engineering and immobilised cultivation are followed to enhance the surfactin production. Rhamnolipid is a secondary metabolite produced under growth limiting condition. However carbon source is excluded out of growth limiting substrate. In the production of rhamnolipid N and P are highly limited compound. However nitrogen source in the form of nitrate shown to increase biosurfactant production. The main carbon sources used for rhamnolipid production are glucose, glycerol, n-alkanes, ethanol and glycerolipids. Ammonia, urea, complex amino acid containing supplement were used as nitrogen sources[21]. In batch cultivation, growth limiting substrates such as plant oil or glucose are used for biosurfactant production. However in glycerol or plant oil serves as a growth limiting substrate for fed batch cultivation. In continuous cultivation mode glucose and hydrocarbon are used as substrates. Camilos-Neto *et al*[22] reported about the application of solid state cultivation in continuous fermentation process. Cooper *et al*[23] reported glucose as a substrate for the production of surfactin, where the product is separated from the reactor by foam fractionation. Noah *et al*[24] studied the application of chemostat with stirred tank reactors for the production of surfactin using *Bacillus subtilis*. Airlift fermentor with continuous collection of foam was used to produce surfactin from *Bacillus subtilis* using potato process effluent as carbon source. Subsequently Yeh *et al*[25] developed a novel bioreactor to avoid the foam spillage during the production of biosurfactant. The application of bubbleless bioreactor using a hollow fiber membrane as an air-liquid contactor was reported to produce surfactin and fengycin by *Bacillus subtilis*[26].

5.2 Factors Affecting The Biosurfactant Production

In the production of biosurfactant various factors affect the yield as shown in Table 2. Some of the important factors are discussed below.

Table 2: Influence of Various Factors on Biosurfactant Production

S.NO	Microorganism	Biosurfactant	pH	Temp.	Carbon source	Yield	References
1.	*Bacillus brevis*		8	33°C	8.5g/l of glucose	-	44
2.	*Pleurotus djamor*	Lipopeptide	5.5	29°C	5g/l of sunflower seed shell	8.9±0.5g/l	45
3.	*Pseudomonas aeruginosa* KVD-HR42	Rhamnolipids	7.8	37°C	23.85g/l karanja oil	5.90±2.1g/l	46
4.	*Bacillus* ICA 56		8.0		Glycerol and sunflower oil	1290mg/l	47
5.	*Pseudomonas aeruginosa* F23	Rhamnolipids	8	30°C	1% coconut oil	2.8g/l	48

5.2.1. Effect of Carbon Sources

Microbes used in the biosurfactant production utilise variety of carbon sources and energy for their growth. *Pseudomonas aeruginosa* utilises water soluble carbon source such as glycerol, mannitol, glucose and ethanol for rhamnolipid production[27]. Among the different carbon sources glycerol behaves differently in such a way that when glycerol concentration is higher than 2%, the rhamnolipid level sharply decreases. Safi *et al*[28] reported that 3% glycerol produce only 2 g/L rhamnolipids with fermentation. He also reported that 6% grape seed oil and sunflower oil also produce 2 g/L of rhamnolipids. In case of 6% glucose, the rhamnolipid yield was found to be 1400-1500 mg/l. It was also observed that 1.3 and 2.1 g/L rhamnolipids were produced with 6% and

5% concentration of diesel and kerosene oil respectively[2]. Soybean lecithin and crude oil were also identified as suitable carbon sources for biosurfatant production. Changjun Zoua[29] proved through his study that soybean lecithin was well utilized for biosurfactant production than crude oil with a slight difference. But crude oil also proved to be an efficient carbon source in case of *Acenitobacter*-related bacteria as reported by Huy *et al*[30]. The use of hydrocarbons such as n-hexadecane and paraffin were also were also attempted as carbon source by Jorge F.B. Pereira and found that only water soluble carbon sources could be easily utilized for biosurfactant production than paraffin and n-hexadecane[31]. However, Onwosi and Odibo[31] suggested that glucose was the excellent carbon source at concentration of 2% for rhamnolipids production and the yield was 5.28 g/l.

5.2.2. Effect of Nitrogen Source

Nitrogen sources play a critical role in biomass growth and thereby the biosurfactant production. *Pseudomonas aeruginosa* was found to be a good strain for the production of biosurfactant. However due to the depletion of nitrogen source it reached the stationary phase which results in reduction of biosurfactant production[33]. Excess of nitrogen source inhibited the biosurfactant producing microorganism hence the production of biosurfactant was found to be less[34]. Several nitrate salts such as sodium nitrate, ammonium nitrate, potassium nitrate was used as nitrogen sources for biosurfactant production and reported. Sodium nitrate was the good nitrogen source for biosurfactant production and the yield was found to be 4.38 g/l[32]. According to Joshi and Shekhawat[35], ammonium nitrate was supported as best nitrogen source for biosurfactant production. Similarly Johnson *et al*[36] reported that potassium nitrate is the better nitrogen source for biosurfactant production by *Rhodotorulaglutinis* IIP-30 when compared to other nitrogen source such as ammonium sulphate or urea. As evaluated by Jorge F.B. Pereira, organic sources such as meat extract and yeast extract could also be efficiently utilized as nitrogen sources and found to affect the biosurfactant production[31].

5.2.3. Effect of Temperature

Temperature is also one of the important factors for biosurfactant production. Rhamnolipid productions increased with temperature from 25 to 30°C and remain constant from 30 to 37°C and slightly decreased when the temperature reached 42°C. Vollbrecht *et al* briefly studied the effect of temperature on the growth of *Pseudomonas aeruginosa* and rhamnolipid production. At higher temperature such as 47°C provided unfavourable condition for the culture growth and hence rhamnolipid production was found to be lesser. Similarly for *Tsukamurella sp.* culture, at higher temperature cell aggregation occurs which results in lower glycolipid production. However certain microorganisms such as *Acinetobacter baylyi ZJ2* could withstand higher temperature (40–45°C) was identified through the investigation carried out by Changjun Zoua. An optimum temperature of 30°C was suggested where cell growth was promoted and yielded a higher glycolipid production. Joice and Parthasarathi also showed that the highest biosurfactant production by *Pseudomonas aeruginosa* PBSCI was at the temperature of 30°C[37, 38, and 31].

5.2.4. Effect of pH

pH is another important factor which affects the biosurfactant production[39]. A pH range of 6.0-6.5 was found to be ambient for the biosurfactant production. At pH above 6.5, the biosurfactant production was found to be decreased. At pH 4 - 4.5, the bacterium was unable to reduce the surface tension of culture medium therefore yield of biosurfactant tends to decrease. Cooper and Goldenberg[18] reported that an increase in pH from 6.5 to 7.0 has not decreased the growth of microorganism for biosurfactant production. However lowering the pH affected the biosurfactant production[40]. Similarly above pH 7, the growth was retarded in an alkaline environment and was reported by Changjun Zoua while studying biosurfactant production using *Acinetobacter baylyi ZJ2*[29]**.** pH was found to affect the metabolism of microorganisms[40]. Joice and Parthasarathi[37] studied the biosurfactant production by altering the pH from 5.0 to 8.5 and observed that surface tension reduction of 29.19 mN/m at pH 6.5 and emulsification activity was 75.12% at pH 7.0. Joice and Parthasarathi[37] concluded that biosurfactant production by *Pseudomonas aeruginosa* PBSC1 was maximum at pH 7.0.

5.2.5. Effect of Aeration and Agitation

Aeration is related to foam accumulation[41]. Agitation affects both mass transfer of oxygen and medium components. Hence aeration and agitation need to be considered an important factor for cell growth and biosurfactant production especially for aerobic organisms. Sen optimized the air flow rate using response surface method as 0.75 vvm for biosurfactant production. Similarly the effect of agitation was studied and reported that an increase in agitation rate from 50 to 200 ppm increased the growth rate from 0.2 to 0.72/ hour and a maximum biosurfactant yield of 80% could be achieved at this condition[42]. This is because the increase in agitation rate greatly affected the dissolved oxygen level in the system from 0.1 to 0.55 mg/l. Hence at higher dissolved oxygen levels, the cell growth was greatly influenced and thereby higher biosurfactant production[43].

VI. Purification Methods for Biosurfactants

In conventional methods, hydrochloric acid in concentrated form was used to extract crude biosurfactants from microbial biomass. However in the present stage, various techniques have been developed to isolate and purify crude biosurfactant such as membrane based techniques, foam fractionation, extraction, adsorption[49]. Membrane separation was first reported by Sen and Swaminathan[49] for the recovery of surfactin. Presently the bubbleless membrane bioreactor has been successfully developed for biosurfactant production[50]. In bubbleless membrane bioreactor the microfiltration and ultrafiltration are coupled together to increase the efficiency of separation process. Foam fractionation is a method for the separation of biosurfactant where acidified hydrochloric acid is added to precipitate biosurfactant. The precipitates can be extracted with solvent[51]. Davis *et al*[52] reported that foam fractionation is an integrated system for the isolation of surfactin.

Nowadays extraction is gaining much attraction towards the researchers due to the easier operation. Various solvents such as chloroform, methanol, ethyl acetate, di-chloromethane, butanol, pentane, hexane, diethyl ether, isopropanol, acetic acid are used for the extraction of biosurfactant. In solvent extraction, hydrophobic moieties are found to be soluble in some solvents which help in extraction of crude product[2]. In adsorption and desorption process, the amberlite XAD 2 or polystyrene resins are used for purification of biosurfactants. During this process, the recovery of biosurfactant is governed by various factors such as agitation rate, activated carbon particle size, pH, temperature, initial adsorbent concentration, amount of adsorbent and ionic strength. In newly developed techniques polymer resins are used to adsorb biosurfactant and for desorption, the organic solvents are used. The active carbon is used as an adsorbent for recovery of surfactin[53]. Further the regenerated activated carbon also can be used for the recovery of biosurfactants[54].

VII. Analytical Methods

Several analytical methods have been utilized and reported by many researchers in their analysis for characterisation of biosurfactant. Table 3 indicates the biosurfactant type, microorganisms, solvent and type of analytical method.

Table 3: Type of Biosurfactants, Bacteria, Solvent and Analytical Methods Involved

Biosurfactant& Bacteria	Analytical Method	Chemicals/Solvents required	Reference
Rhamnolipids *Pseudomonas aeruginosa*	HPLC	CH_3CN-H_2O	55
	TLC	$CHCl_3/CH_3OH/CH_3COOH$	56
	TLC	CH_3OH/H_2O	57
Pseudomonas fluorescens	TLC	CH_3CN/H_2O	58
P. aeruginosa MTCC 2297	HPLC	CH_3CN (Contain 2- bromoacetophenone and triethylamine)	59
Lipopeptide *Acinetobacter baylyi* ZJ2	FTIR	$CHCl_3/CH_3OH/CH_3COOH$	60
Sophorolipid *Candida bombicola*	HPLC with ELSD	CH_3CN/H_2O	61
Phospholipid *Acinetobacter sp.*	GC-MS	$CHCl_3/CH_3OH$ (Extraction Method)	62
Trehalose lipid *Rhodococcus sp. P32C1*	HPLC	CH_3CN	63
Surfactin *Bacillus Subtilis ATCC 21332*	HPLC	CH_3CN/TFA	64

VIII. Application of Biosurfactants

Biosurfactants in Metallurgical Industry

Nowadays, various pollutants are released in to the environment due to vast industrialization. One of such pollutants is heavy metals released from metallurgical industries. Heavy metal being a toxic pollutant contaminates soil, water and seems to get accumulated into food chain. Heavy metals are persistent in nature and cause serious environmental issues. Techniques such as excavation have been reported to clean up the soil contaminated with heavy metal and disposal of contaminated soil to the land sites[65].

In bioreduction of these heavy metals, Microbes can be used as a whole cell biocatalyst to transform the metal into various states[66]. Soil washing and soil flushing is well known bioremediation method to treat heavy metal contaminated soil using biosurfactants. . In an in-situ bioremediation, the biosurfactant are charged on the soil using the drain pipes and trenches[67]. However in ex-situ, the soil is collected from the location and transported to wash column and washed with biosurfactant solution. Biosurfactant could greatly improve the solubility of heavy metals at high concentration and critical micelle concentration. A strong ionic bond is developed between positively charged metal and negatively charged surfactants and finally a surfactant-metal complex is formed. By lowering the surface tension the metal-biosurfactant complex is desorbed from the soil. Generally the solubilisation of metals using biosurfactant is referred to as bioleaching, a process describes as dissolution of metals from mineral source by certain naturally occurring microorganism or from their products.

Biosurfactant converts solid metal into soluble form. The mechanisms such as binding, complexation, desorption and precipitation may found to occur in the removal of heavy metals. Precipitation of heavy metals in water has been practiced as an important method of treatment in industrial wastewater for many years. A combined method of biosurfactant precipitation with chemical treatment techniques such as ion exchange has been reported to be effective in heavy metal removal.

Di-rhamnolipids produced from *Pseudomonas aeruginosa* have been used in the immobilisation of metals from multi-metal contaminated soil[68]. They are also used in the removal of various heavy metals such as chromium, lead, cadmium and copper from soil. Marine biosurfactants are typical type of biosurfactants isolated from marine bacterium used in the remediation of polyaromatic hydrocarbon[69]. However no study report is found for heavy metal remediation. The biosurfactant synthesized from marine organism has the capability to chelate toxic heavy metals. Therefore it is used in the treatment of heavy metal containing waste water. Addition of alkali enhances removal of heavy metals[70]. Foam technology is another advancement method in the biosurfactant based bioremediation. Wang and Mulligan investigated the performance of rhamnolipids to remove Cd and Ni from sandy soil. Generally the foam formed flows into a porous medium and made more uniform and hence makes an efficient contact with metals. The bare rhamnolipid solution used in the removal of Cd and Ni has an efficiency of 61.7% and 51%. But rhamnolipid coupled with foam found to enhance the efficiency of Cd and Ni removal with 73.2% and 68.1%[71]. Massara *et al*[72] investigated on the removal of Cr (III) from kaolinite contaminated with chromium. The factors such as pH and addition of NaOH could positively affect the metal removal. The chelating action of biosurfactants was greatly enhanced by pH and hence higher metal removal. The addition of NaOH increase the biosurfactant solubility thereby promotes better metal removal[47]. The removal of heavy metals reported by different authors is shown in Table 4.

Table 4: Removal of Heavy Metals by Biosurfactant Producing Organism

S.NO	Metals	Microorganism	Removal (%)	Reference
1.	Cr	*Pseudomonas aeruginosa*	46	73
		Aspergillus niger	21-36	74
2.	Cd	*Bacillus strain* H9	36	75
		Aspergillusterreus	70	76
		Pseudomonas aeruginosa	73.2	71
3.	Cu	*Thiobacillus ferrooxidans*	25	77
		Schizosaccharomyces pombe	11-25	78
4.	Pb	*Pseudomonas aeruginosa* PU21	80	79
		Aspergillus niger	13-88	74
5.	Ni	*Pseudomonas spp.*	98	80
		Candida spp	29-57	81
		Pseudomonas aeruginosa	68.1	71

Biosurfactants in Petroleum Industry

Biosurfactant producing organisms (indigenous or injected) are exploited in oil recovery in oil producing wells. By direct injection of nutrients with microbes that are capable of producing desired products for mobilization of oil, by injection of a specific microorganism or injecting biosurfactants through this method, the microbial enhanced oil recovery process is implemented. Interfacial reduction of tension/oil viscosity, reservoir repressurizations are followed by this process. By injection of biosurfactants, bacteria such as *Pseudomonas aeruginosa*, *Bacillus licheniformis* and nutrients, the oil recovery was showed to be increased by 30-200%[82]. Microbial enhanced oil recovery is the best method to recover oil from high viscosity crude oil or from reservoirs with low permeability. Oil field emulsions are one of the major problems for the petroleum industry. It occurs at various stages while processing the crude oil. To control oil field emulsion, the de-emulsification process is one of the best methods to recover oil from these emulsions. A conventional de-emulsification process is obtained by centrifugation, heat treatment and chemicals. Biosurfactants have the ability to retrieve the use of chemical de-emulsifier insitu and it can provide eco-friendly solution. Some of the bacterial species such as *Acinetobacter* and *Pseudomonas* species are the main de-emulsifiers in the mixed cultures[83]. To disrupt the emulsion, the microorganisms exploit the amphiphilic nature of biosurfactants or hydrophobic cell surface. The classes of biosurfactants such as glycolipids, glycoproteins, phospholipids and polysaccharides are the microbial tools to displace the emulsifiers from the oil- water interface[1]. Biosurfactants having potential application to recover oil from petroleum tank bottom sludges and facilitates heavy crude transports through pipelines. From the used oil sorbents the soaked oil can be removed with the help of rhamnolipids[43]. Main factors such as sorbent pore size and washing time are affecting the oil removal. By using the commercial rhamnolipids 95% oil removal was achieved. Apart from using crude biosurfactant, the application of fermentation broth could effectively remove crude oil from contaminated sites as well as motor oil by 85% and 90% repectively[47]. The rate of oil recovery reported by different authors is shown in Table 5.

Table 5: Recovery of oil by Biosurfactant Producing Organism

S.No	Biosurfactants Producing Organism	Biosurfactants	Biosurfactant yield	Recovery of Oil from Oil Contaminated Soil (%)	Reference
1.	*Bacillus subtilis* CN2	Lipopeptide	7150mg/l	84.6 ± 7.1	84
2.	*Bacillus subtilis* BS-37	Surfactin isoform	585mg/l	96	85
3.	*Bacillus* strain		Crude BS 0.081- 1 g/l CMC Value19.439mg/l	30.22 – 34.19	86
4.	*Bacillus subtilis* B 30	Surfactin	Crude BS 0.3 – 0.5 g/l CMC Value 1:8	17-26	87
5.	*Candida sphaerica*	Anionic biosurfactants	4.5g/l	75 (Clay soil) 92 (Silty Soil)	88
6.	*Candida tropicalis*		3.61± 2.1	78 - 97	89
7.	*Candida glabrata* UCP 1002		7.52g/l	92.6	90
8.	*Candida sphaerica* UCP 0995	Biosurfactant Lunasan	9g/l	95	91

IX. Conclusion

In this review paper, the various perspectives of biosurfactants are consolidated into fine and simple concepts for the readers to understand easily. In general, this paper summarizes the need of biosurfactants for the environmental application to harness the eco-friendly natural process and to catalyze the production rate. The in-depth study has led to the development of various strains for the large scale production of biosurfactant and some of the screening techniques have been included for identifying the BS producers. Various operational parameters affecting the production process are also well explained. Analytical techniques such as HPLC, TLC, GC-MS, foam fractionation, membrane separation etc. were discussed briefly for the purification of the product. Various operational parameters affecting the production process are also well explained. Finally, in the application part, the role of biosurfactant in oil and metal related industries are also discussed.

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