

A Review on Recent Advances in the Application of Biosurfactants in Wastewater Treatment

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ABSTRACT

Microorganisms produce a variety of non-conventional surface-active molecules, known as biosurfactants. The biosurfactants find diverse applications in the oil industry, agriculture, emulsifiers, and wastewater treatment, to name a few. Since they are produced from microbes, advantages such as biodegradability, lower toxicity, and environmental compatibility can be leveraged compared to the chemical surfactants. Recently, biosurfactants found potential usability in treating wastewater generated from various domestic, industrial, and agricultural sources. The application of biosurfactants in wastewater treatment is mainly due to their excellent foaming ability, specific activity, and high selectivity under wide operation window of temperature, pH, and salinity. Within the broad field of wastewater treatment, biosurfactants are used as vesicle forming materials in sludge treatment, separation of oil-water, and microbial growth enhancers. The continued research on biosurfactant seeks readily-available renewable resources for biosurfactant production and applying in complex wastewater generated by various industries. Wastewater treatment with use of low cost bio surfactant is one of the important suitable goal in treating the wastewater. The major salient feature of bio surfactant is it replaces the chemical surfactant, produced from the natural sources. It is also important to note that there will be less damage of environment and feasible to use at the industrial scale. This review focuses on the recent developments in biosurfactant production using waste materials and their application in wastewater treatment processes such as contaminant degradation, oil-water separation, heavy metal removal, and effluent flotation.

Keywords: Biosurfactants; Wastewater treatment; Bioremediation; Oil-water separation; Microbial surfactants

1. Introduction

Rapid industrialization severely damaged the aquatic environment by introducing various contaminants into water bodies [1]. In addition, an indiscriminate exploitation of natural water resources for industrial purpose has drastically deteriorated fresh water availability. An uncontrollable trend in population growth has further declined availability of water causing severe water scarcity. Further, inadequate treatment of effluents and improper management of sludge/solid have resulted in the discharge of plethora of contaminants and pollutants into fresh water bodies [2]. As a result, water bodies have become a hot-spot and dumping yards of chemicals flowing out through industrial effluents [1]. Additionally, the demand for new materials has created a new kind of water pollutants named “emerging contaminants” which pose severe threat to aquatic and human life. United Nations World Water Development Report 2017 mentioned that over 80% of wastewater is discharged into water bodies without proper treatment [3]. Wastewater is generated by various industries such as textile, tannery, food processing, petroleum and petrochemicals, fertilizers, etc. It is due to a persistent demand for chemicals by an industrialized world that caused a significant stress on natural bodies.

Various chemicals such as personal care products, pharmaceuticals, dyes, petrochemicals, food additives, and other organic compounds present in industrial effluents. Most of the cases, the contaminants entering into water regions are hydrophilic in nature. However, the presence of hydrophobic compounds, such as petroleum residues, aromatics, chlorinated organics, fatty acids, and oils and greases, in industrial effluents is inevitable. They not only damage nature of water bodies but also inflict a severe threat to environment as well as aquatic and human life [4].

Hydrophobic organic compounds (HOCs) in effluents contribute to the continuous deterioration of the natural aquatic environment. The disposal of HOCs into the aquatic environment significantly affects the quality of water bodies, in turn, aquatic life and human beings [5]. HOCs such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated bisphenyls (PCBs), hydrocarbons, and their derivatives are highly toxic and potentially cause damage to the aquatic environment and human beings. The hydrophobic moiety of HOCs keeps their bioavailability at lower levels [6]. Hence, treatment processes for contaminated water containing HOCs impose challenges for its degradation or removal at a satisfactory level. Traditionally, surfactants have been utilized in wastewater treatment plants to remove HOCs from the effluents. The surfactants



reduce the surface tension between water and non-polar contaminants which, therefore, results in the improvement of the bioavailability of HOCs. However, the employment of synthetic surfactants for the removal of HOCs is still an environmental issue as they are produced from fossil-based resources [7]. Hence, there is an urgent requirement for alternative strategy which is green, facile, cost-effective, and also efficient to address the challenges associated with HOCs removal from water bodies.

The growing concern for sustainable development has tremendously transformed the way industries operate. Replacement of synthetic surfactants by biosurfactants is one of the transforming steps to establish green technologies which rely solely on renewable resources [8]. Bioreclamation of water bodies is not only ecofriendly but also effective in removing or degrading the contaminants. The shift from synthetic surfactants to biosurfactants was initially retarded by the high production cost of biosurfactants [9]. This obstacle was overcome by using organic waste materials as substrates to grow microorganisms that produce biosurfactants. The valorization of low-value organic materials has subdued the disposal of waste produced from various industrial sources [10]. The strategy provides valorization to organic waste and replaces synthetic surfactants for the removal of HOC from water environments.

A very few reviews have been conducted on biosurfactants as surface active agents which talk about different sources of substrates and various methods of production and isolation. However, it is observed that, no review has been conducted on the employment of biosurfactants as wastewater treatment agents. The current review is the first of its kind to discuss the exploitation of biosurfactants for the treatment of wastewater to encourage green technologies as well as to replace conventional surfactants which are synthesized from petrochemicals and pose severe threat to environment. The review presents the characteristics of biosurfactants, mechanism of their interaction with wastewater and pollutants, substrates employed for the production of biosurfactants, methods and techniques used for growing biosurfactant producing microorganism and for the isolation of biosurfactants from the media. Majorly, it discusses about their efficacy for the degradation and removal of pollutants from wastewater.

Initially, the review paper discusses about the properties of biosurfactants which attribute to the wastewater treatment characteristics. Further, it explicitly explains the mechanisms of interaction between biosurfactants and wastewater for the removal of pollutants along with a discussion on

performance defining parameters such as emulsification index, critical micelle concentration, and surface tension. The synthesis of biosurfactants from various sources, organic (including waste material) and inorganic, is extensively reviewed and presented here for the benefit of researchers to understand the scope of biosurfactant production on industrial scale and to provide insights into the development of the technology. In addition, studies are conducted to summarize the performance of biosurfactants in order to degrade contaminants and to remove pollutants such as heavy metals, and lipophilic components from wastewater generated from agricultural, industrial and domestic activities. Finally, the limitation of biosurfactants in the wastewater treatment is exclusively presented to inspire the researchers to widen the role of biosurfactants for the wastewater treatment processes as a green technology.

1.1. Properties of surface-active agents

Hydrophilic-lipophilic Balance (HLB): It is indicative of hydrophilic or lipophilic characteristics of the surfactant. HLB scales from 0 to 20 units and the surfactant's nature depends on these values. Surfactants with HLB values in the range of 3.5 to 6 have a stronger affinity towards water-in-oil (W/O) emulsions. In contrast, for oil-in-water (O/W) emulsion, the HLB values are higher, ranging from 8 to 18. HLB values are determined empirically or based on the molecular structure of the emulsion. HLB values help in the selection of the emulsification system for the surfactants to be as emulsifiers, foaming agents, detergents, and wetting and spreading agents [11].

Critical Micelle Concentration (CMC) of biosurfactant and its importance:

The concentration above which surfactants form micelle is known as CMC. The CMC value of surfactants indirectly reveals the quantity of surfactant required to remediate the contaminant. The lower the CMC, the lower the quantity of surfactant required to form micelle, which increases the bioavailability of the contaminant [12]. Biosurfactants' CMC value decides the pathway through which biosurfactants degrade or remove HOCs from the water entities [13]. **Figure 1** shows the schematic of the surfactant molecule and the formation of micelles above critical micelle concentration and also represents the changes associated with physical properties such as surface and interfacial tension [14].

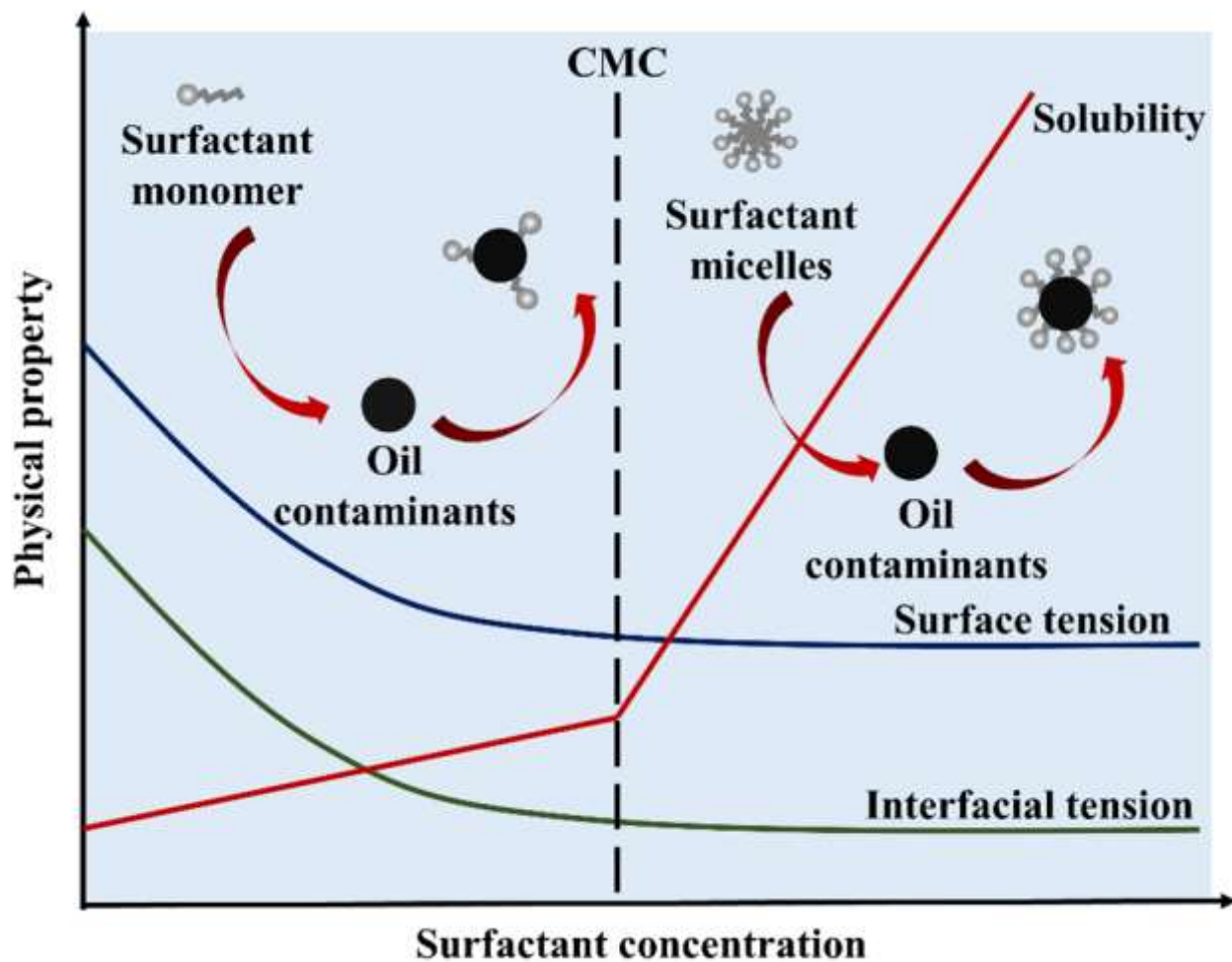


Figure 1: Schematic of the surfactant molecule and formation of micelles above critical micelle concentration. (Reproduced with permission from ref. [14])

1.2. Mechanism of biosurfactant

Biosurfactants improve the surface interactions between polar and non-polar substances by reducing the surface tension at their interaction. The biosurfactants' amphiphilic nature allows them to attach their hydrophobic tail to non-polar compounds and hydrophilic head to water at the interface. Hence, the bioavailability of HOCs improves to a satisfactory level upon which removal or degradation is possible. Biosurfactants improve the bioavailability of HOCs in a water system by three pathways: mobilization, solubilization, or emulsification [12].



Mobilization: the phenomena will take place when the concentration of biosurfactant is below its CMC. At these conditions, the biosurfactants reduce surface tension (ST) between two opposing entities at their interface. This will improve the interactions at the surface due to the amphiphilic nature of biosurfactants [15]. Therefore, bioavailability will increase in such a way that the contaminant can be easily removed or degraded as shown in **Figure 2**.

Solubilization: When the concentration levels are greater than CMC values, biosurfactants start to form micelles with HOCs. Micelle is an associated structure of biosurfactants and non-polar compounds formed within a polar environment. The hydrophobic tails of biosurfactants create a compatible environment inside the micelle where hydrophobic molecules can be trapped. The polar heads of surfactants can be open towards the water environments [12]. The formation of micelles, vesicles, and bilayers at the concentrations beyond CMC drastically increases the bioavailability of HOCs.

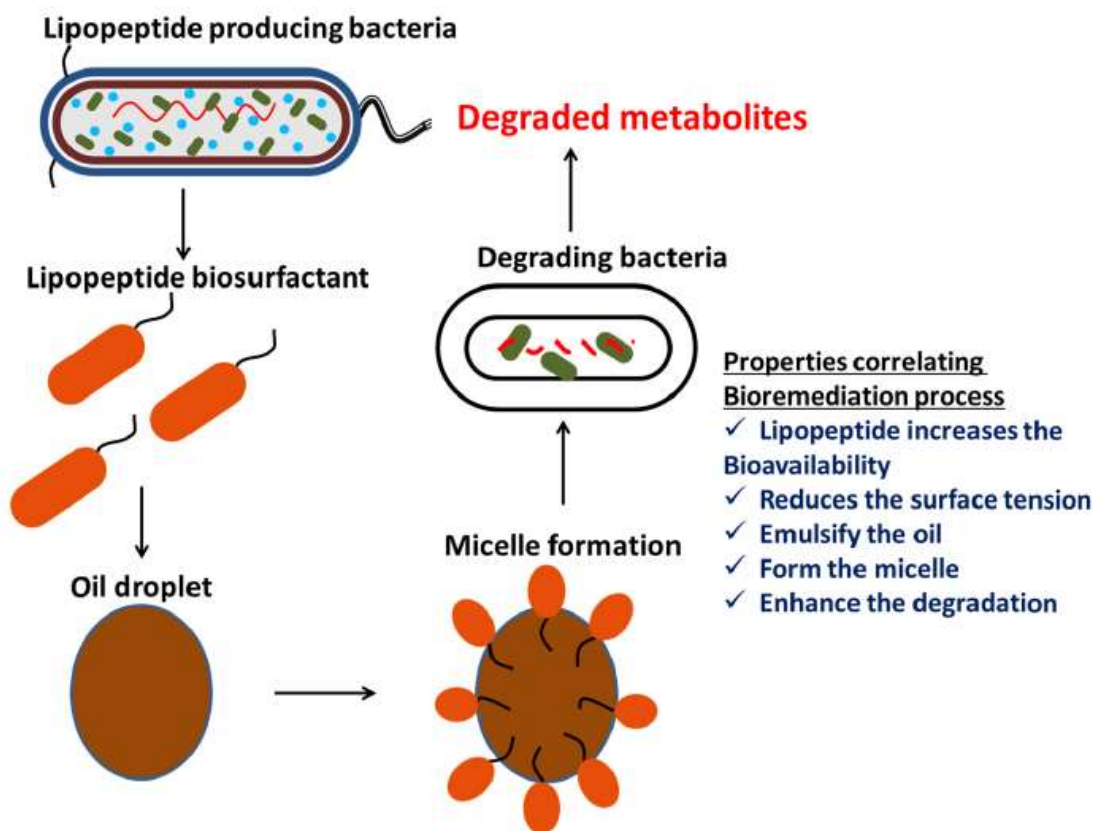


Figure 2: Interaction of biosurfactant with an oil droplet, an HOC (reproduced with the permission from ref. [16])

Emulsification: the dispersion of HOCs in the water phase as tiny droplets is enhanced by the presence of biosurfactants in the mixture. Like mobilization and solubilization, biosurfactants' emulsification also improves the bioavailability of the non-polar compounds in the polar world. Among all biosurfactants, the high molecular weight biosurfactants are the most effective emulsifying agents [17].

1.3. Defining Parameters

Surface Tension: the primary objective of supplying biosurfactants to the polar/non-polar mixture is to reduce water's surface tension. The reduction in surface tension enhances the interaction between two opposing entities. The supplied biosurfactants pull the hydrophobic and hydrophilic compounds together at their interface and make them available for the degradation or removal from the water bodies [5].

Emulsification Index (EI₂₄): is the index that defines the degree of ability of biosurfactants to form emulsions of hydrophobic moieties in hydrophilic environments. The emulsification nature of biosurfactants is more for greater values of EI₂₄, which is generally expressed in percentages. The values of surface tension and EI₂₄ after supplying the biosurfactants to the oil-water mixtures reveals what kind of mechanism biosurfactants initiate to make contaminants available for their degradation [18].

1.4. Classification

Biosurfactants are classified as low molecular weight and high molecular weight based on their size. Carboxylic acids, fatty acids, amino acids, and sugars constitute low molecular weight biosurfactants, whereas lipopolysaccharides, heteropolysaccharides, lipoproteins, and their derivatives make high weight biosurfactants molecules. Glycolipids, composed of a saccharide and a non-saccharide, are the most widely studied and commercialized low molecular weight biosurfactants. The low molecular weight biosurfactants are further classified into cationic, non-ionic, anionic, and amphoteric based on their net charge. In general, low molecular biosurfactants improve the surface activity, and high molecular weight molecules are supplemented for emulsification [19]. The diversity of biosurfactants is the result of their chemical structures. The structural composition of biosurfactants allows them to find applications in various pharmaceuticals, oil and petroleum, agriculture, and food industries. The biosurfactants are



majorly composed of proteins, glycolipids, lipopeptides, and fatty acids [20]. The biosurfactants are mainly classified based on size (low molecular weight, high molecular weight), composition (glycolipids, lipopeptides, fatty acids, phospholipids, polymeric surfactants, particulate surfactants), and charge (anionic, cationic, amphoteric).

2. Lipid- and Fatty Acid-Based Biosurfactants

2.1 Glycolipids: These are the most common biosurfactants with a definite structure and ability to reduce the interfacial surface tension. Glycolipids are low molecular weight compounds and are covalently bonded with carbohydrate as well as mono/disaccharides with long-chain fatty acids. Glycolipids form an integral part of the cell membrane, which influences the transport of material across cell membranes. The microbial outer layer is activated by the different compounds of microorganisms, which makes them permeable [21]. They have a hydrophilic carbohydrate head and a fatty acid hydrophobic tail, which impart amphoteric nature. The most important candidates of glycolipids are Rhamnolipids, Sophorolipids, and Trehalolipids [22].

Rhamnolipids: They are the anionic counterparts of glycolipids. Several studies have been carried out on the use of rhamnolipids for bioremediation. Rhamnolipids were first reported by Jarvis and Johnson in 1949 and were the first biosurfactant produced by *Pseudomonas aeruginosa* [15]. The Rhamnolipids are formed by the combination of one or two rhamnose molecules (hydrophilic) linked with the fatty acid (hydrophobic). The fatty acids can be saturated or unsaturated, depending on the alkyl group chain [23]. Rhamnolipids typically have low molecular weight, and they have great applications in food processing, medical care, and the agriculture sector. The production of rhamnolipids requires a high energy feedstock (Carbon source). The fermentation process is highly efficient to produce rhamnolipids with low economical feedstock as an energy source. The rhamnolipids are mainly produced from the *P. aeruginosa* by the fermentation process coupled with foam fractionation. The nitrogen source is released from the feedstock that helps the *P.aeruginosa* during the biosynthesis of rhamnolipids [24]. The response surface methodology (RSM) was introduced to maximize the rhamnolipids production from waste fry oil by fermentation broth and the activation of surface properties for rhamnolipid biosurfactant by acid precipitate. RSM predicted the highest production of 6.2 g/L rhamnolipids. The fermentation reactor required 20 g/L frying oil, 9.7 g/L glucose, and 1.78 g/L of ammonium nitrate [25].



Sophorolipids: These are the commercially used glycolipid biosurfactants produced from several species of yeast from food waste. They possess hydrophilic and lipophilic characteristics due to the presence of disaccharide sophorose and the long-chain hydroxylated fatty acid in their structure. The saccharides are biologically converted from vegetable oils and waste cooking oils for the production of sophorolipids [26]. The potential species of yeasts studied for the production of sophorolipids are *Starmerella bombicola*, *Candida bombicola*, *Candida stellate*, *Candida apicola*, *Wickerhamiella domercquae*, *Torpus bombicola*, *Candida floricola* obtained different sources [27]. However, several yeast species, such as *Candida albicans* and *Candida glabrata* [28], are recently studied to produce sophorolipids optimally.

The production of the biosurfactants from the waste oil cake decreases the cost of the final product, and they are environment friendly. The extraction of sophorolipids from oil cakes can be done by fermentation of winterized oil cakes [29]. The production of sophorolipids by the food waste is by using yeast of *Starmerellabombicola*. The primary collection from the restaurant food waste is for the enzyme hydrolysis. The hydrolysis process takes place in the presence of three types of enzymes; *Bacillus subtilis* as protease, *Aspergillus niger* as lipase, and glucoamylase. The enzymatic hydrolysis of yeast containing waste food is achieved by growing yeast in the nutrient media in a batch fermentation process. This hydrolysis process enhances the productivity of yeast as well as Sophorolipids [26].

Trehalolipids: Mainly marine bacterium, belonging to Actinobacterial genera Mycobacterium, Gordonia, Dietzia, Tsukamurella, and Williansia, produce trehalolipids. Rhodococcus extracted from seawater are trehalolipids, which show good emulsifying characteristics in the pH range 2-10 and temperatures from 20 to 100°C [30]. The production of trehalolipids involves the aromatic, aliphatic, and some of the components of carbon [31]. Trehalolipid, which is extracted from the emulsified fermentation process, is economically feasible and environment-friendly. Microbial diversity increases the environmental aspects to develop the new structure of the microbial component [32].

Fatty acid-based biosurfactants: microorganisms produce fatty acid-based biosurfactants through the biochemical pathway of alkane oxidation. The fatty acids are responsible for reducing the surface tension between polar and non-polar compounds. Yeast, fungi, and several bacteria produce these fatty acid-based biosurfactants by extracellular activity [33]. Among all the



commercial biosurfactants derived from the forest, agricultural and biological materials, fatty acid-based biosurfactants constitute the largest part. Corynomycolic acids are the strongest biosurfactants under the fatty acid category. Corynomycolic fatty acids with chain lengths of C12 – C14 have been observed to be the strongest surface activity improving agents [34]. **Figure 3** shows the chemical structures of few common biosurfactants.

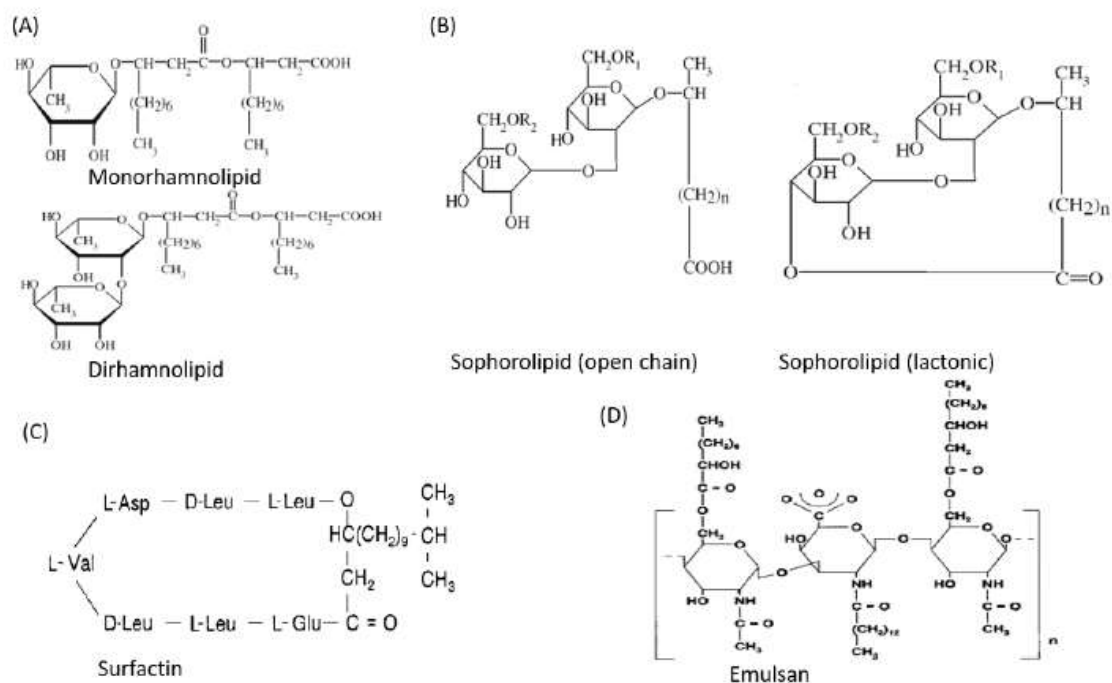


Figure 3: Chemical structures of biosurfactants (Reproduced with permission from ref. [35])

Fatty acyl groups, known as the hydrophobic building blocks of biosurfactants, are used either in the form of fatty acid esters or free fatty acids, which are the oleochemical byproducts to produce biosurfactants. Ester groups present in fatty acid-based biosurfactants couple the lipophilic and lipophobic moieties. Ester groups make them biocompatible and promote the biodegradability of biosurfactants. Hence, they are widely used in pharmaceuticals, cosmetics, personal care products, and food derivatives. Yet, the applicability is limited due to their instability. The fatty acids are subjected to a reduction upon which fatty amines or fatty alcohols are formed that increase the stability [36].

Coconut oil, palm kernel, and palm stearin are rich in high-lauric oils, which is an excellent source for the production of fatty acid biosurfactants. A fall in the availability of high-lauric oils through

vegetative sources has initiated a hunt for alternative green sources. Scientists have identified that algal oils have great content of high-lauric oils. In addition, it is noticed that the metathesis of olefins, a rearrangement reaction of segments of alkenes by removing and renewing double bonds between carbon atoms, produces fatty acids or alkyl esters of fatty acids [37].

3. Biosurfactants from Waste Materials

The commercialization of biosurfactants applications is limited by substrate cost and operating cost for the microbiological culture. Researchers have identified different types of waste materials as the potential to produce certain types of biosurfactants as they are enriched with glucose, nitrogen, and other nutrients such as phosphorous, iron, manganese, and magnesium, which are required for the growth of surfactant producing microbes [38]. Using agro-industrial wastes as a culture medium for microbes for producing biosurfactants can reduce the production cost by 10% [39]. In recent years, the concern for the safe environment has tremendously increased. The disposal of waste material derived from various sources is a challenging problem. The valorization of waste as a substrate for the production of biosurfactants is a potential solution to overcome waste disposal problems [40]. Researchers have utilized different waste materials for the production of biosurfactants for their targeted applications.

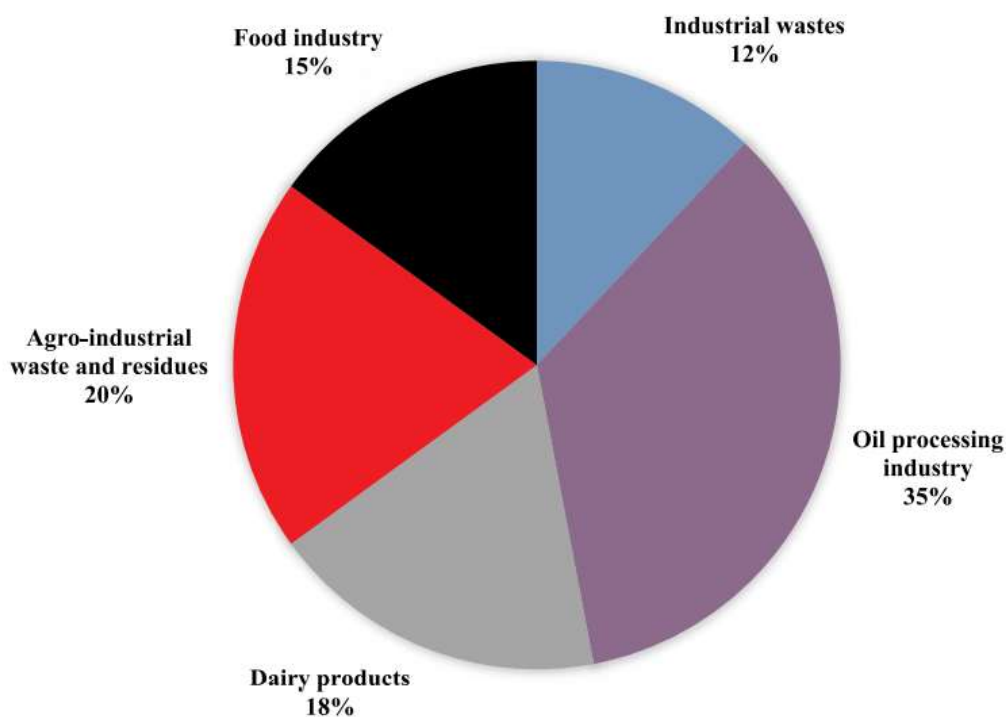


Figure 4: [Contribution of various types of waste materials used for production of biosurfactants](#) (reproduced with the permission from ref. [41]).

Microorganisms use organic matter and other nutrients present in the waste material for their growth and proliferation. Through dissimilation reactions, the microbes break-down carbon present in the waste matter and derive the required energy by the metabolic activities. The simultaneous catabolic and anabolic activities of microorganisms present on the substrate results result in the extracellular production of biosurfactants. The characteristics such as surface tension, EC_{24} , and CMC of biosurfactants produced using waste materials as the culture media have shown similar characteristics as the biosurfactants produced on synthetic culture media [18]. **Table 1** shows biosurfactants' production from various sources, their characteristics, and their performance in wastewater treatment.

3.1 Food and Agricultural Waste as substrates

Waste material that generates from the food and agro-industries and their related products are rich in carbon source. Hence, this type of material provides a nutrient-enriched environment for the growth of microorganisms [42]. Researchers have investigated the ability of various food and agriculture-based waste such as sugarcane molasses, bagasse, banana, orange and potato peels, waste cooking oil, frying coconut oil, moringa, and cassava residues to produce biosurfactants [43]. Various types, such as rhamnolipids, sophorolipids, lipopeptides, mannosylerythritol lipids, are extracted from low-cost raw materials: distillery waste, food and vegetable process effluents, molasses, rapeseed oil, cassava flour wastewater, frying coconut oil, chicken feather peptones, coffee wastewater, etc. [44]. The type of biosurfactant produced depends on the substrate used as its composition facilitates the growth of microbial species in the fermentation process [9].

Studies on several *Lactobacillus* strains for the production of biosurfactants using sugarcane molasses (cellulosic sugar) and glycerol as substrates found that the type of surfactant biomolecules produced from these bacteria as glycolipids and glycolipopetides in the form of multicomponent mixtures. One such production method, proposed by Rodrigues et al., with few alterations [45]. The production took place in a lab-scale bioreactor with MRS growth medium added with varying concentrations of lactose as substrate. The results show that the yield of production using sugarcane molasses and glycerol is more than conventional synthetic broth. The

product recovery from the broth could be extracted by using the solvent method [46]. The yield of 2.43-3.03 g/L was obtained by using sugarcane molasses as a substrate. The yield in this study is comparatively higher than the previous studies suggest that the supplementation of broth with extracts of yeast and peptone increases the yield of biosurfactant production. The drop collapse test, surface tension, and emulsification indices were the key parameters used to characterize the biosurfactant [47].

Corn steep liquor (CSL) can act as a potential substrate for production of biosurfactants from *Bacillus subtilis* #573. Growing these bacteria on a CSL culture medium as the carbon source could yield 1.3 g/l of biosurfactant, as reported [34]. Supplementing the growth medium with metals, such as iron, manganese, and magnesium, enhance this yield to 4.1, 4.4, and 3.5 g/l, respectively. The sulfates of these metals act as cofactors for enzymes responsible for surfactant production by cells. The surfactants derived from *Bacillus Subtilis* have a better performance in oil recovery or bioremediation applications than chemical surfactants [48]. Waste cooking oil (WCO) and Coffee wastewater (CW) can also be used as an economical substrate for biosurfactant production from bacterial strains isolated, such as *Pseudomonas Aeruginosa*. Such biosurfactants have the capacity to reduce surface tension from 50 to 30 mN/m [35]. These microbes were isolated from the pinyon rhizosphere, out of which the maximum biosurfactant yield of 3.7 g/l was obtained [42]. The biosurfactants produced from these strains were identified as glycolipids based on Thin Layer Chromatography (TLC). The biosurfactants have shown an EI₂₄ of 58%. 3.55 g/L of rhamnolipids were synthesized using WCO, collected from a coconut oil mill with an emulsifying activity of 71.7% [40]. The biosurfactant yield of 8g/l has also been reported from the growth of *Paenibacillus sp.* on waste sunflower oil and coconut oil as a low-cost substrate [49].

Bacillus subtilis ANR 88, grown on agro-based waste materials such as extracts from orange peels, banana peels, potato peels, whey, molasses, and bagasse, producing an amount of 0.089, 0.049, 0.032, 0.241, and 0.127 g/L were produced, respectively. Biosurfactants produced from molasses culture medium are useful in synthesizing gold and silver nanoparticles [50].

Waste frying oil as a carbon source, chicken feather peptone as a nitrogen source, and KH₂PO₄ were used as a culture medium for the production of rhamnolipid biosurfactant. The optimal concentrations of carbon source, nitrogen source, and KH₂PO₄ were defined using Box-Behnken design as 52, 9.2, and 4.5 g/L, respectively. The optimal conditions produced 13.31 g/L



rhamnolipid with an emulsification activity of 80% was achieved [18].

3.2. Industrial Waste as Substrates

Crude Glycerol: Trans-esterification of waste vegetable oil produces biodiesel. The residue obtained from this process is known as crude glycerol. Crude glycerol was used as a substrate for the production of biosurfactants. After 72 h incubation period, strains derived from crude glycerol have shown a reduction in ST from 69 to 30 mN/m. The EI₂₄ of biosurfactants produced is 26% [51]. The schematic representation of production of lipopeptide biosurfactant from oil source can be seen in **Figure 5**.

Wastewater: extensive research has been conducted to study the biodegradation of hydrocarbons, particularly phenanthrene, by using biosurfactants isolated from a creek, which was polluted with multiple industrial wastewaters. These biosurfactants were resistant to heavy metal and were found to be effective in the removal of crude oil and phenanthrene with a minimum removal percentage of 94 and 85, respectively [52]. Food processing industrial wastewater was used as a potential source for biosurfactant production. These biosurfactants have degraded 91% of oil and grease coming from electronic industry effluent in 12 days of incubation period [53]. Researchers extracted 667 bacterial isolates from the Stellenbosch wastewater treatment plant in South Africa. 32 of these 667 isolates were identified as biosurfactants as they have reduced the surface tension of the medium from 71.1 mN/m to 32.1 mN/m [54]. It is observed that the biosurfactants produced from vineyard pruning waste (VPM) were different as the carbon source was changed. Glucose from VPM as a substrate produced glycolipopeptide, whereas glycoprotein biosurfactant was produced with lactose as a carbon source. Further observation revealed that the emulsifying ability of biosurfactants and their chemical structures is affected by the extraction process. This variation is a sign of broader applications of biosurfactants in industries [55].



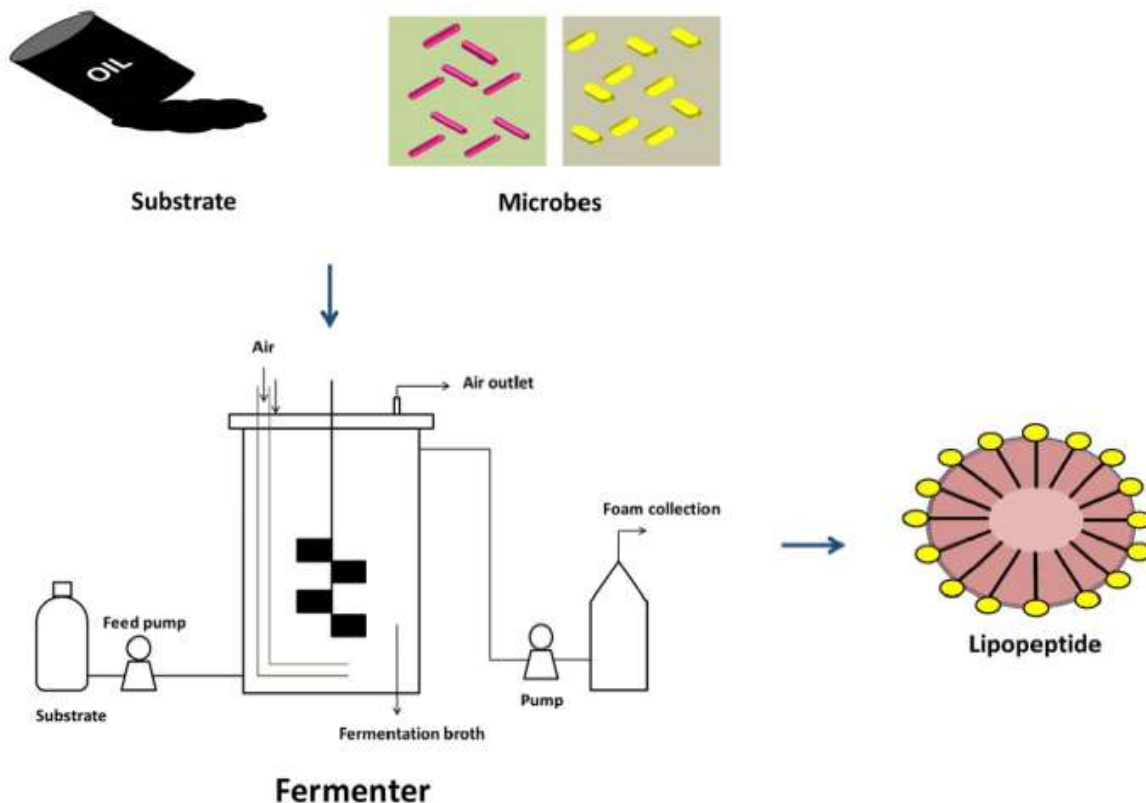


Figure 5. Production of lipopeptide biosurfactant from oil source (Reproduced with the permission from ref. [16])

Animal Waste: the literature deals with biosurfactants derived from animal waste constitutes 7% of the literature available on biosurfactants produced from renewable resources [9]. Meat processing industries produce byproducts such as lard and animal fats, which can be used as a substrate for microorganisms to produce biosurfactants since the market for animal fats is replaced by vegetable oils [56]. Actinomycete strains which were capable of producing biosurfactants were isolated from animal fat waste, which was collected from the local market, as a substrate. A yield of 46.23% suggests that industrial-scale production is possible [57]. The maximum production of biosurfactants produced by *C. bombicola*, which was grown on animal fat, was observed after an incubation period of 68 h [20]. It was observed that the surface tension of the broth was reduced to 27.6 mN/m. Certain type of biosurfactants has derived from fish waste with an EI_{24} of 87.6% and a surface tension value of 27.8 mN/m [58]. Wastewater generated from the swine industry is one of the major environmental problems in various counties. Anaerobic digestion of swine wastewater to produce energy through resultant biogas can be a potential solution. But the

digestion was impotent in the removal of ammonia from the wastewater. The removal efficiency of ammonia using biosurfactant produced from the same wastewater was 89% [59].

3.3. Contaminated Soil as Substrate

Biosurfactant produced from *Pseudomonas aeruginosa*, isolated from a soil contaminated with hydrocarbons, is useful for the degradation of PAHs present in the soil [60]. The biosurfactants produced by the microorganism were found to be rhamnolipid, with 55.6% of EI₂₄ and CMC of 60 mg/L. Hydrophobic volatile organic compounds (VOCs) were used to produce biosurfactants to enhance the n-hexane, a hydrophobic VOC, degradation in the contaminated soil [61]. The presence of biosurfactant decreased the ST by a maximum of 10.14%. It has shown 70% of n-hexane removal from the contaminated soil in 120 h. Engine oil contaminated soil was chosen as a substrate for the production of biosurfactants to improve the rate of oil recovery. The biosurfactants were glycolipids that had a capacity of ST reduction up to 30.8 mN/m with an EI₂₄ of 90% [62]. Further analysis, such as the drop collapse test, oil displacement activity, also confirms that the produced biosurfactants can be used in commercial applications.

Table 1: Production of biosurfactants from various sources, their characteristics and performance in wastewater treatment

No.	Source	Bacterial strain	Yield (g/L)	Surface tension reduction	EL ₂₄ %	CMC (mg/mL)	Performance	Reference
1	Sugarcane molasses	<i>L. plantarum</i> G88	2.43	70 to 47.50	49.89			[47]
		<i>L. delbrueckii</i> N2	3.03	70 to 41.90	81.00			
		<i>L. cellobiosus</i> TM1	2.79	70 to 44.20	63.50			
2	CSL	<i>Bacillus subtilis</i> #573	1.3	70 to 31.80	54.50 to 59.00			[39]
	WCO	<i>P. aeruginosa</i> Strain A	3.7		58			
<i>P. aeruginosa</i> Strain B		2.72	50 to 29	-				
<i>P. aeruginosa</i> Strain 83		1.76		59				
3	CW	<i>P. aeruginosa</i> Strain A		No reduction			[40]	
		<i>P. aeruginosa</i> Strain B	3.7		59			
		<i>P. aeruginosa</i> Strain 83						
4	Coconut oil	<i>P. aeruginosa</i> D	3.55		71.7		[63]	
	Molasses		0.241					
	Whey		0					
	Potato peels		0.022					
5	Orange peels	<i>B. subtilis</i> ANR 88	0.089				[50]	
	Banana peels		0.049					
	Bagasse		0.127					
	Waste frying oil		<i>P. aeruginosa</i> OG1	13.31		80		
7	Crude glycerol	Various strains		72 to 28	5 to 26		[51]	
	Lactoserum			72 to 38	44 to 69			
8	Multi-industrial effluent	<i>B. subtilis</i>			2.9 to 3.1		Degradation of crude oil >94 % Degradation of phenanthrene >85 %	[52]



	Food processing effluent	<i>S. marcescens</i> EU555434					Oil and grease degradation 91%	
9	Electrical and Electronic effluent	<i>A. hydrophila</i> KF049214					Oil and grease degradation 100 %	[53]
	Oil palm	<i>B. cereus</i> KJ605415					Oil and grease degradation 100 %	
10	Wastewater treatment plant	Various strains		71.1 to 32.1	90			[54]
11	Vineyard pruning waste	<i>L. paracasei</i>		72 to 20.9		1.18		[55]
12	Animal fat waste	Actinomycetes	46.23					[57]
	Fish waste	<i>C. aquaticum</i>		72 to 27.8	87.6			[58]
13	Bagasse	<i>C. spp</i>		72 to 33.9	61.6			[58]
14	Swine wastewater	<i>P. frederiksbergensis</i>	2.98			32	Ammonia removal 93 %	[59]
15	Contaminated soil	<i>P. aeruginosa</i> PF2	160	73.2 to 30.5	55.6	60	PAH removal 99 %	[60]
16	Contaminated soil	<i>P. sp. Strain</i> NEE2		72 to 40		340	n-Haxane removal 70 %	[61]
17	Contaminated soil	<i>O. anthropi</i> HM-1	4.9	70 to 30.8	90		Oil recovery 70 %	[62]
		<i>C. freundii</i> HM-2	4.1	70 to 32.5	89		Oil recovery 67 %	[62]
18	Synthetic medium	Laccase enzyme					Bisphenol A removal 65 %	[64]
19	Sunflower oil and glycerol	<i>B. licheniformis</i>	7.8	72 to 48	62.5		Triclosan removal 47.2%	[65]
							Dye removal improved by 10%	[66]
20	Vineyard pruning waste	Lipopeptide biosurfactant					Sulphates removal improved by 62%	[66]
21	Cassava wastewater	<i>B. subtilis</i> LB5a		72 to 25.97	61 % in gas, 74 % in diesel	28.33	Soluble COD removal 80 % Oil and grease removal 70 % Production of methane	[67]

4. Applications to Wastewater Treatment

4.1. Contaminant degradation

Rhamnolipid type biosurfactants are effective in enhanced removal of Bisphenol A from wastewater by laccase. Bisphenol A is commonly found as a pollutant in industrial wastewater and have adverse effects on human health [64]. These Biosurfactants in wastewater medium prevent loss of enzymatic action for degradation compared to the mixtures of other synthetic surfactants, resulting in the removal of Bisphenol A by 65%. Liu et al. simulated the molecular mechanisms for phenols' interaction with Laccase in the presence or absence of Triton X or rhamnolipids by molecular docking and molecular dynamics. The hydrogen bonding and van der Waals interaction bind phenol with the enzyme, and surfactants change the enzyme conformation resulting in phenol degradation. Rhamnolipids have a strong influence than Triton X for enzymatic degradation of phenolic compounds in aqueous solution [68]. Rhamnolipids produced from *Stenotrophomonas* sp genus, isolated from polluted river water, have also shown the capability to reduce different PAHs, total petroleum hydrocarbon (TPH), and phenolic compounds from petroleum wastewater in the range of 70 to 90% [69]. The degradation of phenols is primarily due to enhanced enzyme activity of phenol hydroxylase, catechol 1,2-dioxygenase, and catechol 2,3-dioxygenase in the bacterial species, followed by a further reduction in surface tension and increased bioavailability of hydrocarbons.

Triclosan's antifungal and antibacterial nature makes it to use extensively in personal care products, such as soap, toothpaste, detergent, body wash, mouth fresheners, and perfumes. From domestic wastewater, removal of 47% triclosan was achieved in two hours of processing by a lipopeptide biosurfactant produced by *Bacillus Licheniformis* [65]. Bioremediation of triclosan is difficult because of its less bioavailability. Aerobic degradation by rhamnolipids is another method to remove triclosan from water-sediment systems, reported the processing time of 56 hours with di-Rhamnolipid with 93.87% biodegradation efficiency [70].

A biosurfactant produced from corn steep liquor has been shown to increase the ability of vineyard pruning waste that contained hydrogel. The modified bio-composite has promoted a 10% increase in dye removal and a 62% increase in removing sulfates from wastewater [66]. The characterization of extracted biosurfactant suggests that these eco-friendly bio-molecules have

great potential in contriving green adsorbents to the industries. Industrial dyes are toxic, and their discharge into wastewater without treatment can cause serious health effects as they are also carcinogenic [71]. Iron oxide nanoparticles, functionalized with rhamnolipid biosurfactant produced from *Pseudomonas aeruginosa*, is beneficial in photocatalytic biodegradation of methylene blue dye by adsorption process, in comparison to bare iron oxide nanoparticles, with the removal efficiency close to 93% [72].

Highly contaminated effluent from poultry wastewater having soluble chemical oxygen demand (SCOD) and OG (Oil and Grease) concentration can be treated by biosurfactant surfactin produced by *Bacillus subtilis* LB5a. Anaerobic treatment with biosurfactant could remove over 80% SCOD and 71% OD at optimal conditions from wastewater [67]. Similarly, rhamnolipids produced by *Pseudomonas aeruginosa* were applied in enzymatic anaerobic treatment of poultry wastewater and reported the removal of 95.8% OG [10]. Bovine bile, derived from meat processing industries, as a biosurfactant having high CMC values, has also been reported to degrade the high concentrations of fats, oils, and grease in poultry waste with 100% recovery of bile [73].

Ammonia from the anaerobic digestion of swine wastewater in China was treated with a biosurfactant isolated from microorganisms grown on the same wastewater. It was treated with multiple soil layer bioreactors fed with biosurfactants to remove the ammonia from the effluent. It was noticed that the removal of ammonia was improved to 93% [59].

4.2. Oil-Water Separation

Oil spillage, accidents in oil and fuel cargo transportation, and effluents from petroleum, oil, and food industries cause severe damage to water bodies. They are insoluble, inflammable, corrosive, and hazardous. The hydrocarbons, oily in nature, are responsible for causing severe threats to the natural environment: alkanes, phenols, cycloalkanes, aromatic hydrocarbons, and BTEX (benzene, toluene, ethylbenzene, and xylene) [17]. Hence, removal of the oily substances from water is a seriously concerning issue to mitigate their effect on the environment and life.

Partial fossil fuel combustion, petrochemical industrial activities, oil spillage, and biomass burning generate carcinogenic polycyclic aromatic hydrocarbons (PAHs). The degradation of PAHs by a pool of biosurfactants isolated from the consortium has been investigated [74]. It is observed that the concentration of PAHs was increased in the aqueous phase, which resulted in increased

bioavailability and degradation. Their further study revealed that as the ring number of PAHs increases, the degradation decreases.

Rhamnolipids have been extensively studied in the bioremediation of contaminants in water and soil systems. Operating parameters such as pH and ionic strength of the solution influence the performance of rhamnolipids [20]. *P.aeruginosa*, a biosurfactant producing bacteria, is used to degrade the oil contaminants from marine water extracted from Brazil seawater [75]. It is observed that the performance of biosurfactants is better at pH 8, with a dispersion index of about 100%. The decontamination of oil substances from marine water is observed via solubilization and mobilization of hydrophobic substances. In a recent study, the application of biochar from sugarcane residue, Rhamnolipids, and nitrogen in bioremediation of Total petroleum hydrocarbons (TPH) in coastal soil, contaminated with crude oil reports 80% degradation in 20 days of incubation period [76].

Fats, oils, and greases (FOGs) form soap-like deposits with other substances present in the effluents. These deposits block the flow, and the layer formed by these HOCs deter the oxygenation phenomena. Food industries produce substantial amounts of FOGs. The HOCs in effluents generated from food industries primarily constitute animal fats and vegetable oils [77]. Experiments on biodegradation of FOGs such as butter, vegetable oils, animal fats, and margarine using biosurfactants were conducted [78]. The preliminary degradation studies were conducted by quantifying oxygen consumption and carbon-dioxide evolution. Except for butter, the other compounds were degraded by the biosurfactants effectively. The removal efficiency was enhanced by adjusting pH; at pH 8.5, 80% removal of animal fat was observed, and 72% removal of pig lard was noticed at pH 8.

4.3. Heavy Metal Removal

The extensive use of heavy metals in industrial processes causes long-term ill effects on the environmental ecosystem. The major sources of heavy metals are mining operations, metallurgical and milling processes, electronic products, and various other processes such as electrolysis, electro-osmosis, electroplating, and tanneries industries. This issue attracts the attention of researchers because heavy metals cannot be degraded or destroyed. Microorganisms can change the speciation of heavy metals and transform them into nontoxic form. Junior et al. conducted a

study on the washing of soil contaminated with Zn, Cu, and Pb as heavy metals with yeast-derived biosurfactant. The biosurfactant, produced from *Candida tropicalis* with molasses, frying oil, and cornsteep liquor in distilled water as the growth medium. 30 to 80% of zinc, and copper removal is achievable within 30 mins of serial washing in the column [63]. Recently, biosurfactants extracted from *Ziziphus Spina-Christi* are used to prepare a colloidal solution, removing Pb^{2+} and Cu^{2+} from aqueous solutions [79]. *Ziziphus Spina-Christi* is a plant whose leaves produce saponins as a biosurfactant, is generally used in making shampoos and detergents [80].

Moreover, the incorporation of electrokinetic techniques with biosurfactants can enhance heavy metal removal from the sludge [81]. Electrokinetic treatment reduces the labile speciation of heavy metals and increases stable speciation. The authors report that the combination of biosurfactant and electrokinetics technique improves the cumulative electro osmotic flow and the electrolyte electrical conductivity. In another innovative attempt, researchers modified the surface of ground grass using rhamnolipids [82]. This biosurfactant modified green grass is able to remove 85% of Cd^{2+} at pH 7 from an aqueous solution. Wang et al. functionalized two-dimensional Ti_2CT_x MXene with biosurfactants and used it to remove Pb(II) ions from the solution [83]. The investigation indicated that biosurfactants enhance spacing between the nanosheets, increasing the active adsorption sites and ions exchange efficiency for Pb(II). **Figure 6** shows the schematic indicating steps for preparing biosurfactant-functionalized MXene and adsorption mechanism for Pb(II). Removal of heavy metals from soil using biosurfactant mainly includes three steps. When soil is washed with biosurfactant, the molecules of biosurfactants are adsorbed at the interface of heavy metal and wet soil. Then, micelles of biosurfactant encapsulate the heavy metal through electrostatic interactions. Lastly, membrane separation can be used to recover the biosurfactant. **Figure 7** shows the mechanism of heavy metal removal from the soil using biosurfactant.



Figure 6: (A) preparation process of biosurfactant-functionalized MXene and (B) the schematic of the adsorption mechanism for $Pb(II)$. (Reproduced with permission from ref. [83])

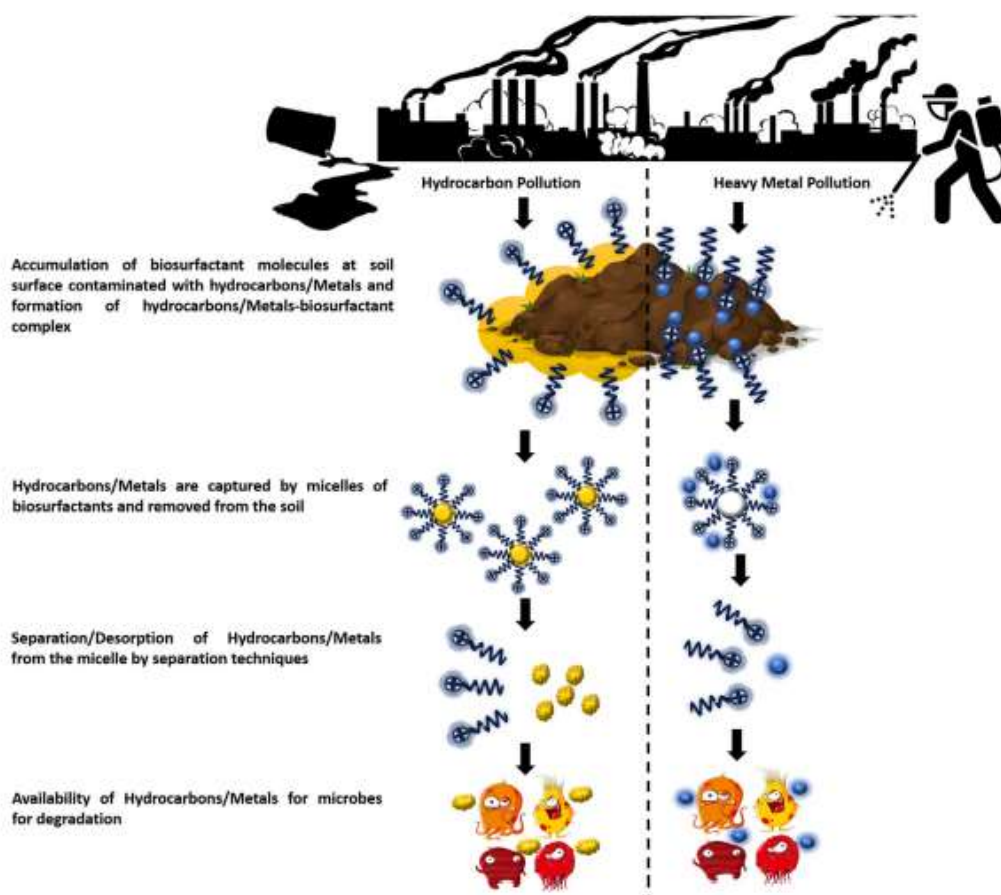


Figure 7: Schematic of a process of heavy metal and hydrocarbon removal from the contaminated soil using biosurfactant (Reproduced with permission from ref. [84])

4.4. Flotation of Effluents

Flotation is a simple but important separation process employed for the removal of metals, oils, and different types of effluents in wastewater from various industries [85]. It works on the principle of gravitation and agglomeration of colloids in aqueous solutions. Air bubbles rising through the column agglomerate the contaminants in wastewater, which rises due to density difference and float on the surface, known as dissolved air flotation. In most applications, synthetic surfactants facilitate the collection of agglomerates at the surface of the liquid. However, the possible toxicity of the collectors raises concerns about the sustainability of the technique. Studies on applying biosurfactants as potential collectors positively improve the sustainability of the process. In a study, two types of biosurfactants, tea saponin, and rhamnolipid, replaces chemical surfactant (Triton X-100) to control bubbles hydrodynamics in the flotation process [86]. A small amount of tea saponin can reduce the aspect ratio of a single bubble by 33%, decrease the terminal velocity of the bubble by 35%, and narrows the amplitude of the bubble trajectory by 27% due to the Marangoni effect. Moreover, biosurfactants increase the number density of bubble swarms and decrease bubbles' size, thereby increasing the specific surface area of bubbles. Zhao et al. employed the tea saponin to selectively remove polyethylene from a ternary plastic mixture of polyethylene, acrylonitrile-butadiene-styrene (ABS), and thermoplastic rubber (TPR) in a flotation process [87]. During the flotation process, ABS and TPR selectively get wetted by the tea saponin, which reduced their presence in floated products and allowed only polyethylene to float. The hydrogen bonds between specific plastic and tea saponin provide selective wetting. The evidence of the mechanism was demonstrated by the occurrence of redshift, which indicated that the π -electron density of the aromatic system in ABS or TPR was considered as the main hydrogen bond acceptor, and one of the strong polarity hydroxy in tea saponin was considered as the donor. **Figure 8** schematically illustrates the mechanism of wetting selectivity. Recently, Silva et al. extracted biosurfactant from *Pseudomonas aeruginosa* and used it in dissolved air flotation to remove the oil from the effluent. The biosurfactant enhanced the separation efficiency of dissolved air flotation by enabling the adhesion of the contaminant particles, resulting in 65 to 95% oil separation efficiency [88].

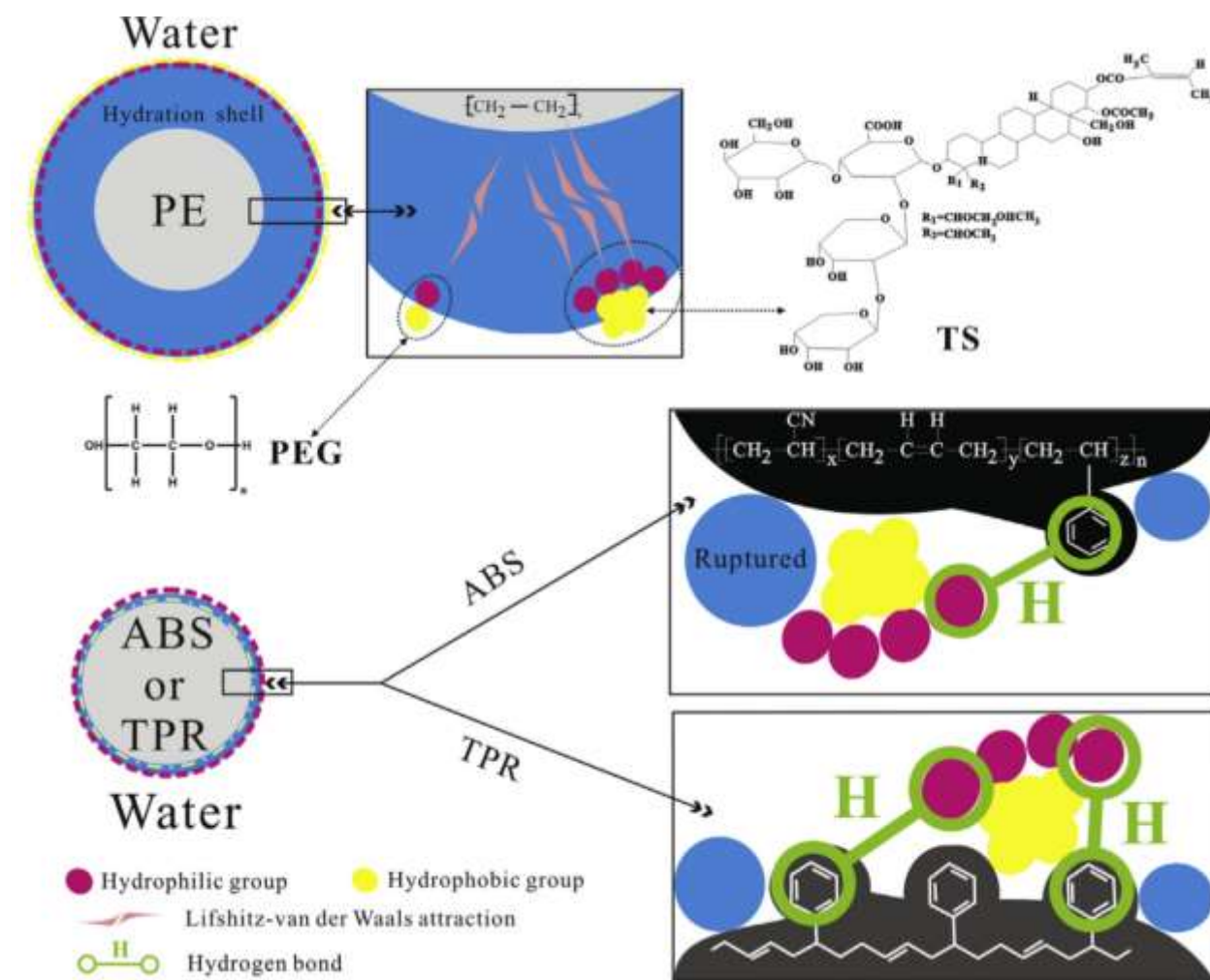


Figure 8: Schematic depiction of the mechanism of wetting selectivity of tea saponin towards acrylonitrile-butadiene-styrene (ABS) and thermoplastic rubber (TPR). (Reproduced with permission from ref. [87])

5. Factors Affecting Wastewater Treatment using Biosurfactants

The previous sections presented different types of pollutants in wastewater from different sources and how biosurfactants isolated from microorganisms show the ability for bioremediation. It is necessary to overview certain factors such as pH, temperature, properties of biosurfactant, biosurfactant concentration, and operating conditions of the process being used that affect the efficacy of a biosurfactant in the removal of contaminants in wastewater. In accordance with the works conducted to study the potential for their removal, several techniques at lab scale have been employed, and parameters were optimized by modeling. **Table 2** shows the basic operating

parameters which can be modified to control the removal/degradation efficiency of contaminants in wastewater using biosurfactants.

Optimization of the process variables such as temperature and biosurfactant concentration can also be done using the Response surface methodology (RSM). RSM uses a collection of mathematical and statistical methods to design experiments and process optimization. Six steps are necessary for the design and optimization of a physicochemical process, as shown in **Figure 9** [89]. In a study conducted for anaerobic treatment of wastewater using a biosurfactant by Cosmann et al., the dependency of biosurfactant concentration in wastewater and the operating temperature on solubilized COD by fitting a non-linear equation was evaluated using RSM [58]. The results predicted high solubilization of COD at temperatures above 60⁰C, resulting in high percentage removal of SCOD. Similarly, application of a biosurfactant produced by *Candida guilliermondi* UCP 0992 to separate oily waters from petroleum wastewater in a batch dissolved air flotation prototype device, whose operating parameters were optimized for oil-water separation using RSM [90]. They expressed the oil removal efficiency of the prototype as the function of biosurfactant flow and effluent flow. On this basis, high flow rates of biosurfactant and lowest for affluent results in 94% of oil removing efficiency of the system. The emulsion liquid membrane (ELM) technology is useful in the removal of heavy metals from wastewaters, in which biosurfactants can be used as a stabilizer of the emulsion. The Mn(II) ion removal process was optimized using Artificial Neural Network (ANN) model by fixing the initial conditions like temperature, pH, and inlet concentrations [91].

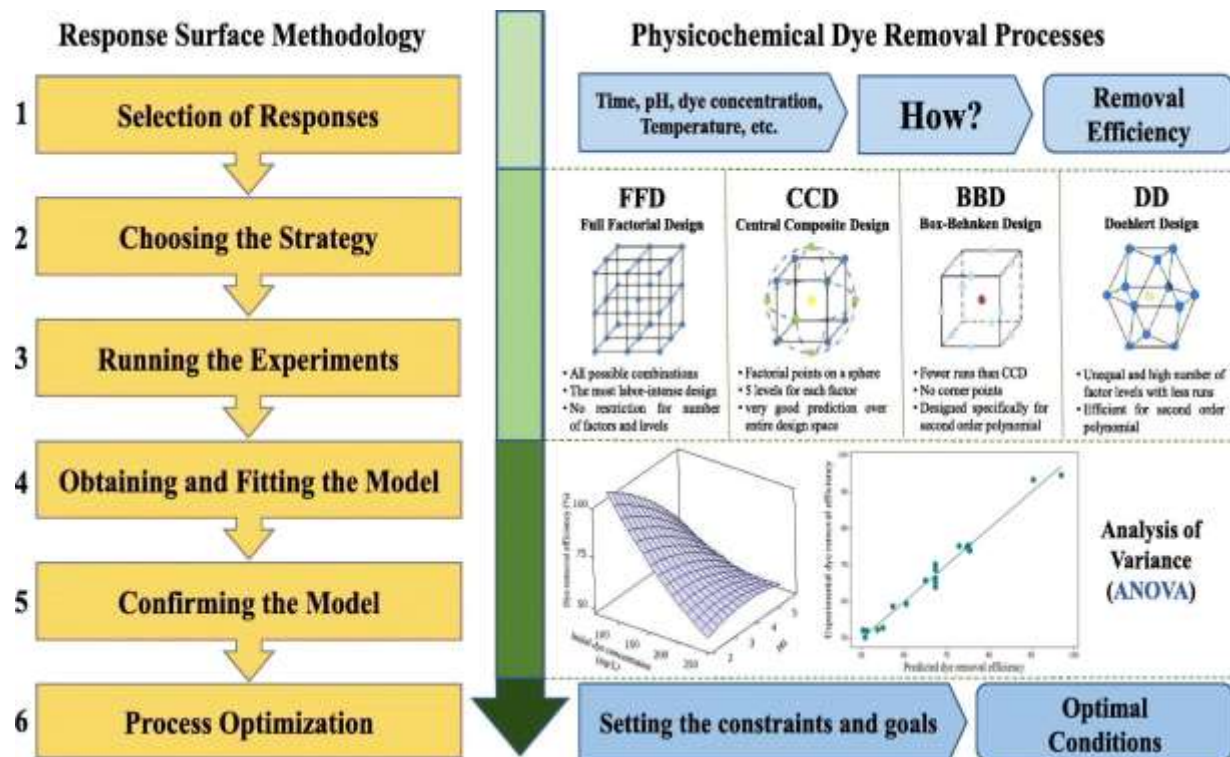


Figure 9: Steps for RSM in the optimization of a physiochemical process. (Reproduced with permission from ref. [89])

Table 2: Typical operating parameters in a bio-surfactant-assisted removal of various contaminants from wastewater

Biosurfactant	Target Contaminant	Removal Mechanism	Operating Factors				Removal Efficiency	Reference	
			Biosurfactant Concentration	pH	Temperature	Contaminant Concentration			Time
Rhamnolipid	Bisphenol A	Enzymatic degradation	1 ppm	5.8	25° C	50 ppm	120 min	65%	[64]
Lipopeptide	Triclosan	Degradation in HPLC	7.8 g/l	--	25°C	0.05 - 5 mg/l	16 h	100%	[65]
Rhamnolipid	Triclosan	Aerobic Degradation	0.125 - 1.25 g/l	6 - 9	15 -35 °C	30 -120 µg/g	56 d	63- 93 %	[70]
Lipopeptide	Dye from winery wastewater	Adsorption on biosurfactant modified biocomposites	399.4 mg/l	--	--	31.5 mg/l	2 h	76%	[66]
Rhamnolipid	Methylene Blue dye	Photocatalytic Degradation using Rhamnolipids functionalized Iron Oxide Nanoparticles	40 g/l	--	--	2.5 mg/100 ml	3 h	92.72%	[72]
Polysaccharides and Proteins	Ammonia	Multiple Soil Bioreactor	0.1 CMC	7.8	--	800-1000 mg/l	>21 d	93%	[59]
Rhamnolipid	PAHs	Degradation in bioslurry reactors	3g/kg	--	37° C	3064 mg/kg	25 d	67%	[74]



6. Economic Feasibility and Recommendations

In general, biosurfactants are considered as one of the prominent bioeconomy products due to their various economic activities [92]. These natural surface active compounds can replace synthetic surfactants as detergents, adhesives, biocides, and for oil recovery and environmental remediation activities [92], [93]. There is a huge demand, worldwide, for surfactants in various above mentioned fields. Due to the depleting nature of fossil-based sources, synthetic surfactants will face an extinction one day. Hence, there exists a great feasibility of production of biosurfactants on large scale as a replacement for the chemical surfactants [36]. From the current review, it can be comprehensible that there is a scope for the employment of biosurfactants in a large scale for wastewater remediation since most of the microbial growth is possible on organic waste and other cheap materials. However, it is lucid that it further requires a great research and time for the industrialization and commercialization. The major concerns regarding the employment of biosurfactant in wastewater treatment methods is their production [94]. The production of biosurfactants is a sensitive with various parameters involved. Providing certain environment for the culture of microorganism requires an intensive care. Further, the isolation of biosurfactants from the medium is a tedious job which often results in contamination to a certain levels. This contamination predominantly effects the performance of biosurfactants as CMC is a function of purity of the biosurfactant [95]. Henceforth, a more investigations have to be conducted by considering the discussed issues to produce biosurfactants commercially.

7. Conclusions and Future Recommendations

Biosurfactants exhibit a combination of physicochemical properties and biological activities. A combination of biological moieties such as peptides, nucleic acids, or sugars with lipids forms hybrid amphiphilic molecules. There are number of potential applications are available for the biosurfactants which includes the food industry, petroleum industries, bioremediation etc. Although microorganisms produce biosurfactants, chemical synthesis can also be used to produce certain biosurfactants. Glycolipids such as rhamnolipids produced by *Pseudomonas aeruginosa*, sophorolipids by *Candida bombicola*, and mannosylerythritol-lipids by *Candida Antarctica* have been chemically synthesized and found extensive use in wastewater treatment. There are very few application are found in the wastewater treatment and treatment of sludge. Despite the advanced research, challenges exist in the form of high production cost and low efficiency of isolation of



biosurfactants. Although this review presented several case studies on the use of biosurfactants for wastewater treatment, a systematic relationship between physicochemical properties and the molecular structure needs to be established. It is also important to note that the biosurfactants will be commercially feasible in wastewater treatment industries if the overall cost will be reduced for the large production scale, it will be feasible if it is attempted with cheap renewable feedstock.

Commercial application of biosurfactants in wastewater treatment can be promoted by furthering the large-scale production of biosurfactants with high purity.

Acknowledgment

This work was supported by the Department of Science and Technology-Science and Engineering Research Board, Government of India [SERB No: IMP/2019/000004].

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