

Eco-Friendly Elixirs: A Critical Review of Biosurfactants for Sustainable Wastewater Solutions

Rushil Bhatt¹, Piyush Shrivastava¹, Jharna Gupta¹, Aditi Vetel¹

Abstract

Biosurfactants are the amphiphilic substances which are acquired from the remains of the living microorganisms and have attracted a lot of scientific interests because of their applicability in various fields. This review aims at reviewing current research and development on biosurfactants with special emphasis on their application in wastewater and oil. Biosurfactants being less hazardous to the environment are produced by microorganisms and works as a substitute for chemically produced surfactants which are dangerous-persistent organic pollutants. One of the primary challenges associated with biosurfactants is the scalability of production and associated costs. Despite these hurdles, the benefits of biosurfactants outweigh the challenges. Their unique properties include stability under extreme conditions of pH and temperature, biodegradability, and improved physicochemical stability. These attributes render biosurfactants particularly suitable for applications requiring environmental compliance and operational versatility. In the context of the oil and gas industry, biosurfactants show considerable promise. They facilitate enhanced oil recovery (EOR) by lowering interfacial tension between oil and water, thereby improving the efficiency of extraction processes. Moreover, ongoing research explores novel biosurfactant types derived from diverse microbial sources, which could further expand their applicability and effectiveness. Interestingly, biosurfactants show potential within the oil and gas sector promising directions within the framework of the novel biosurfactant types based on microorganisms rather than organic or marine origin. Major benefits of biosurfactants, apart from biodegradability and low toxicity, include renewability, stability to extreme conditions of pH and temperatures and improved physicochemical stability. These attributes make them specially suitable to applications which require superior environmental compliance and operational flexibility. Summing up, biosurfactants may be referred to as an endlessly developing branch of biotechnologies and a promising area for the further research and creation of new applications. The diverse applications show the suitability to disrupt sectors dependent on surfactant advancement, prousing environmental solutions following today's policy aspirations. In future research endeavors, it is believed that more advancements will be made that can underscore biosurfactant's efficiency in production, cost effectiveness and their variety applications in technology for a greener world.

Key words: Biosurfactants, microbial surface-active compounds, amphiphilic molecules, alternative substrates

*Author for Correspondence

Rushil Bhatt
E-mail: rushilbhatt-chemengg@msubaroda.ac.in

¹Assistant Professor, Chemical Engineering Department, The Maharaja Sayajirao University of Baroda, Vadodara, India

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INTRODUCTION

The use of natural and environmentally friendly compounds is indeed the need of the day in tandem with the concept of sustainable development. Surfactants are one such group of compounds. The chemical industries of the world have been producing a wide range of surfactants for the last six decades, and some of them belong to hazardous groups of chemicals comprising of toxic compounds, poorly biodegradable compounds, and compounds producing salvaged or non-salvaged

long-term impacts on the environment. Harmful impacts of surfactants (synthetic) on the environment have led to an interest in finding its substitutes, which should preferably be biodegradable and environmentally friendly.[1].

Discovery of biosurfactants, a group of compounds with both hydrophilic and hydrophobic groups produced by microorganisms, have opened up an entirely new and exciting area of research. The search for microbial surfactants began when it was shown that the hydrocarbon emulsifying activity of *Candida lipolytica* was associated with the production of a glycol-lipid

Jimenez et al, (2020) have defined biosurfactants as surface-active compounds synthesized by microorganisms. They have added that the term biosurfactant encompasses all surface active agents produced by microorganisms, including the surface active lipids and hydrophilic surface active biopolymers[2].

Compared with the definition of synthetic surfactants given by Rosen (2012), which states that surfactants are amphiphilic compounds which, when present in low concentration in an interface, alter its surface properties. This is usually expressed as a change in the surface or interfacial tension. Consequently, all biosurfactants can be classified as surfactants, but the opposite is not true. Since surfactants lower the surface tension of a liquid and since lowering of the surface tension is associated with detergency, emulsification, and foaming, it can be said that surfactants are detergency, emulsifying, and foam producing agents. Biosurfactants have properties superior to those of synthetic surfactants in many applications[3].

SOURCES OF WASTEWATER POLLUTION

The sources of wastewater pollution can be various. We have to focus on a certain instances where we are going to have industrial discharges, agricultural runoff, domestic sewage or improper waste disposal which can basically make wastewater in terms of water pollution and harming the environment. UNDP stated in 2017 that the waste water generated is almost $359.4 * 10^9$ metric cube per day [4]. The sources of wastewater and their relevant composition is given as below:

Industrial Discharges

Industries release a wide range of pollutants into water bodies, including heavy metals like cadmium, arsenic, chromium, and lead. These contaminants pose serious risks to aquatic life and human health.

Agricultural Runoff

The excessive use of fertilizers and pesticides in agriculture leads to the contamination of water sources with nitrogen, phosphorus, and other chemicals. This runoff contributes to eutrophication and water quality degradation.

Improper Waste Disposal

Improper disposal of solid waste, particularly non-biodegradable materials like plastics, contaminates water bodies and marine ecosystems. Microplastics, in particular, pose a significant threat to aquatic life.

Domestic Sewage

Domestic wastewater, containing organic matter, microorganisms, detergents, and other pollutants, enters water bodies through sewage systems. This wastewater can harbor pathogens and contribute to waterborne diseases. The industrial sources of waste water pollutants can be described in following figure (Figure 1) as well as the table (Table 1)

The following table (Table 1) showcases the major sources of pollutants found along with their respective pollutants:



Figure 1. Sources of Wastewater Pollution.

Environmental Impact of Untreated Wastewater

As per “The United Nations World Water Development Report 2023: partnerships and cooperation for water” there is significant potential of wastewater as a valuable resource that can address freshwater needs, provide raw materials for energy and agriculture, and reduce environmental impact and health risks when properly treated. The report underscores the importance of improved wastewater management in achieving sustainable development goals (SDG 6), highlighting the need for reducing pollution at the source, reclaiming water, and recovering useful by-products. Additionally, the report mentions the recovery of nutrients like phosphorus and nitrates from wastewater for fertilizer production and the use of treated wastewater for agricultural irrigation and even drinking water supplementation in some cities. Overall, the report advocates for a shift towards viewing wastewater as a valuable resource rather than a mere waste product, promoting sustainable practices for its management and utilization [21].

BIOSURFACTANTS

Biosurfactants are surface-active compounds that are produced by a variety of microorganisms (bacteria, fungi, and yeast) and are unique compared to chemical surfactants in that they are not only more eco-friendly and biodegradable but also have antimicrobial properties. They have characteristics that allow them to be useful to a variety of industries, such as environmental, petroleum, food, and medical, with a potentially wide range in the future. This has led to a drastic increase in research devoted to biosurfactants and their properties since the early 1980s. Due to the large amount of different biosurfactants, their varying properties, and complexity of microorganisms and production, it is apparent that biosurfactants can be an extremely broad and complicated topic. [22,23].

Biosurfactants can be produced by various microorganisms such as bacteria, fungi, and yeast, using substrate as a source of food. The type and range of biosurfactants produced vary among different groups of microorganisms. Generally, biosurfactants are amphiphilic compounds containing both hydrophilic and hydrophobic moieties, which confer surfactant properties to reduce surface and interfacial tensions. This definition encompasses a broad range of compound classes, including phospholipids, fatty acids, neutral lipids, and complex mixtures like lipopolysaccharides and lipoproteins. [24,25].

Table 1. Sources of Wastewater pollutants and their harmful effects.

Pollutants	Sources	Effect	References
Heavy metals like Arsenic, Mercury, Copper, Cadmium, Chromium, and Nickel	Industrial effluents (metal-based, dyes, rubber, etc.)	Accumulation in aquatic organisms and entry in food chain, Severe to chronic diseases possible	[5-7]
Zinc, xylene, benzene, toluene, monocyclic aromatic hydrocarbons, lead, mercury, aluminum, radioactive molecules, platinum group metals, silver, barium, molybdenum, chloramines, radium, fluoride, nonylphenol ethoxylates (NPE), di-(2-ethylhexyl)phthalate, polycyclic (PAH) aromatic hydrocarbons, polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzo-p-furans, nitro musks (chloronitrobenzenes), oestrogenic compounds and polyelectrolytes, etc.	Petroleum, stainless steel, paint industries, power generation, mining, construction, and food industries.		[8-10]
Fertilizer and pesticides, electronic waste containing lead and cadmium, polythene bags, rotten fruits and vegetables, and linear alkyl benzene sulphonates (LAS) and microbes, etc	Kitchen and sewage		[11-12]
Detergents, surfactants	Domestic waste	Foaming of water bodies leading to reduced water supply to aquatic organisms.	[13-14]
Suspended solids and microorganisms	Untreated sewage (human fecal matter + domestic waste)	Reduced sunlight penetration in lakes, etc affecting aquatic flora and fauna. Pathogenic microbes from carriers may cause food and water-borne diseases like cholera, typhoid, etc.	[15]
Pb, Zn	Underground pipes	Cause mental disorders	[16,17]
Nitrates & Phosphates, Pesticides, herbicides	Waste generated from agricultural run-off	Causes eutrophication	[18,19]
Microorganisms (bacteria, protozoa, and viruses)	Animal waste from farms	May cause water-borne diseases	[20]

Contrary to popular belief, surfactant compounds are not restricted to hydrocarbon-solubilizing agents like SDS. All the above-mentioned compound types have demonstrated surfactant properties but are not necessarily deemed as "surfactants" in the conventional sense of the word.[26].

The most common method of biosurfactant screening is the detection of surface activity. [27] (Li Fang et. al., 2023) This involves the measurement of surface or interfacial tensions and the critical micelle concentration (CMC) of the compound in question. Sodium azide can be added to the system to prevent biodegradation of the surfactant, and such experiments are usually carried out at the pure culture stage using the Wilhelmy plate method. High-throughput methods such as the Microbial Adhesion to Hydrocarbons (MATH) technique or the use of a tensiometer can be used at the shake flask stage, and environmental applications may involve simple tests like the oil spreading technique. Measures of surface activity are often taken without any isolation of the biosurfactant, and compounds must be extracted and purified if chemical characterization is to be carried out. This chemical information is essential for the identification of the compound, classification of biosurfactant type, and understanding structure-function relationships.

Types of Biosurfactants

According to Nagtode et.al., 2023 there are different types of biosurfactants been defined by many authors [25] One of the prominent classification is based on structure and microbial origin where they can be classified on the based on their diversity of hydrophilic headgroup or hydrophobic headgroup and their respective charges and other classification is based on types of microbes involved in their production

Classification Based on Structure [28-32]

Chemical Structure: Biosurfactants can be classified based on the diversity of their structures, which include hydrophilic groups containing acids, peptides, cations, anions, mono-, di-, or polysaccharides, and hydrophobic groups comprising unsaturated or saturated hydrocarbon chains or fatty acids. The solubility of biosurfactants in water is determined by their hydrophilic component, while their capillary action is influenced by the lipophilic component.

Classification Based on Microbial Origin [33-37]

Biosurfactants are produced by a variety of microorganisms, including genera like Clostridium, Brevibacterium, Pseudomonas, Rhodococcus, Acinetobacter, Bacillus, and more. Different species within these genera produce various forms of biosurfactants, such as glycolipids, lipopeptides, fatty acids, and polymeric biosurfactants, each with distinct molecular weights and properties. Figure 2 Table 2.

Production of Microbial Surfactants

Batch culture fermentation has been the most widely used method. The microorganisms are placed in a liquid growth medium to which a carbon source is added (glucose, sucrose, etc.). The global objective is to transfer as much carbon as possible into the desired product. Once the carbon source has been depleted, the cell's metabolism is inactivated, ceasing product formation. The derivative from this method often produces undesirable secondary metabolites, is expensive, and not to mention time and labor-consuming.

Table 2. Biosurfactant from different microbial sources and their subcategories.

Biosurfactant	Subcategory	Producing Microbial Genus	Reference
Glycolipids	Sophorolipids	<i>Candida Torulopsis</i>	[39]
	Trehalose lipid	<i>Rhodococcus, Tsukamurella Arthrobacter</i>	[43]
	Rhamnolipids	<i>Pseudomonas, Renibacterium</i>	[38,41,42]
	Liposan	<i>Candida</i>	[44]
Lipopeptides	Surfactin	<i>Bacillus</i>	[40]
Phospholipid and Fatty Acids		<i>Corynebacterium, Micrococcus, Thiobacillus, Asperigillus</i>	[45,46]

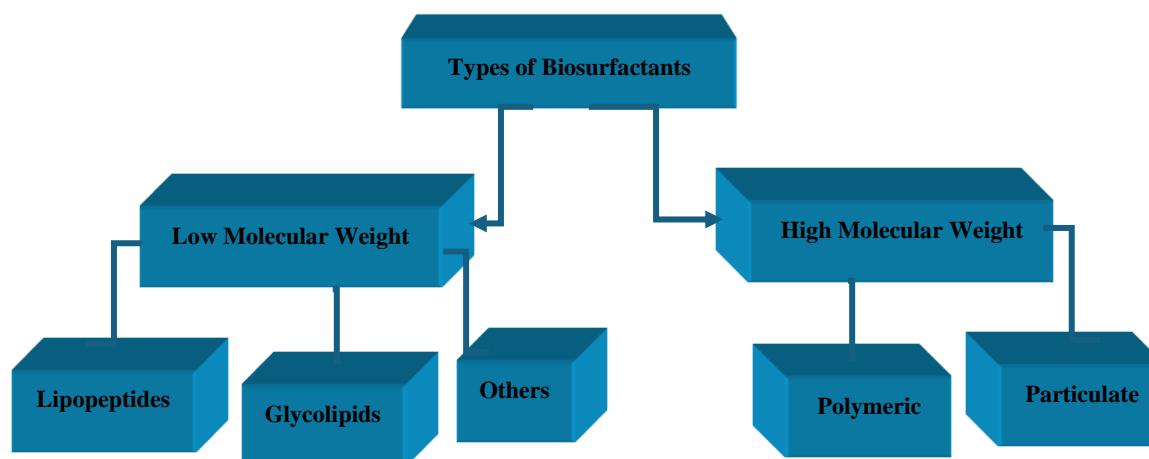


Figure 2. Classification of Biosurfactants.

The upside to using batch fermentation is that the medium is easily optimized and small-scale trials can be done. These trials would be useful in determining the most cost-efficient way to manipulate the medium towards producing a higher yield of the desired product. This could then be scaled up to run large trials.

In terms of wastewater treatment this can be considered in tertiary treatment or bioremediation where the microbes or microbial strains are basically put into or they are reacted under certain condition in different types of waste water to remove the pollutant the pollutant may be heavy metal or organic compound or any biological compound like oil

Optimization of Biosurfactant Production

Optimizing the production of biosurfactants is crucial for enhancing their yield and efficiency in various industrial applications. By understanding the factors that influence biosurfactant production and employing optimization techniques, researchers can maximize the output of these environmentally friendly molecules.

Factors Influencing Biosurfactant Production

Microbial Strain Selection

Different microbial strains, such as *A. niger*, *Bacillus*, *Streptomyces*, and others, exhibit varying capabilities in biosurfactant production. Selecting the most efficient strain is a critical factor in optimizing biosurfactant yield.

Carbon Source

The choice of carbon source significantly impacts biosurfactant production. Substrates like waste frying oil, lactose, yeast extract, and industrial by-products such as molasses and corn steep liquor have been used to enhance biosurfactant yield. Table 3.

Table 3. Produced Biosurfactant and their waste substrates.

Biosurfactant Type	Producer Microbial Strain	Low Cost or Waste Raw Material	Ref
Rhamnolipids	<i>Pseudomonas aeruginosa</i> AT10, <i>Pseudomonas putida</i> 33 and 300-B	Soybean oil refinery wastes, Soybean Waste frying oil, agroindustrial waste	[49,52]
	<i>Pseudomonas aeruginosa</i> BS2	Curd whey and distillery wastes	[53]
	<i>Pseudomonas aeruginosa</i> DS10-129	Sunflower and soybean oil	[54]
	<i>Pseudomonas aeruginosa</i> 47T2 NCIB 40044	Waste frying Soybean and olive oil	[47]
	<i>Pseudomonas aeruginosa</i> LBI	Soybean and sunflower oil soap stock waste	[56]
	<i>Pseudomonas fluorescens</i>	Vegetable oil	[57,58]
	<i>Pseudomonas</i> sp. DSM 2874	Rapeseed oil	[60]
	<i>Pseudomonas fluorescens</i>	Olive oil	
	<i>Pseudomonas aeruginosa</i> DSM 2874	Crude glycerol (biodiesel production)	[61]
Soporolipids	<i>Candida bombicola</i> ATCC 22214	Turkish corn oil	[63]
	<i>Candida antarctica</i> and/or <i>Candida apicola</i>	Oil refinery wastes	[65]
	<i>Candida lipolytica</i> IA 1055	Babassu oil	[68,69]
	<i>Candida glabrata</i> UCP 1002	Cassava flour wastewater	[67]
Lipopeptide	<i>Serratia marcescens</i> , <i>Arthrobacter</i> sp. N3	Sunflower oil	[62,66]
	<i>Bacillus subtilis</i>	Corn steep liquor, Potato process effluents	[48]
	<i>Bacillus subtilis</i> ATCC 21332, <i>Bacillus subtilis</i> LB5a	Olive oil	[50,51,55]
Mannosyl erythritol lipid	<i>Candida</i> sp. SY16	Soybean oil	[70]
Phospholipid	<i>Klebsiella pneumoniae</i> WMF02	Sludge palm oil	[71]

Cultural Conditions

Parameters like pH, temperature, agitation rate, and incubation period play a vital role in biosurfactant production. Optimal cultural conditions need to be identified to maximize biosurfactant output.

Based on the experiments conducted where there was some carbon sources and number of other supplements to increase surfactant yield with respect to temperature, pH, time and agitation. These are tabulated in Table 4.

The optimum range for Rhamnolipids was found in temperature to be 27 degrees centigrade to 37 degree centigrade where the medium and the supplement was usually mineral as well as basal. The time taken was one of the most widely distributed from 48 hours to 288 hours and the pH medium was around 7 to 7.5. The agitation speed required was also larger from 120 RPM to 330 RPM

In comparison sophorolipid had only basal salt medium with temperature just from 27 to 30 degree centigrade. The pH range was 5.6-6 and the time taken was comparatively the least to all others from 72 hours to 168 hours and the agitation speed required was also comparatively lesser than rhamnolipids at 150 RPM to 200 rpm.

For lipopeptides like surfactin which was taken in mineral salt medium as well as different different chemicals where it was found that the temperature requirement is larger than sophorolipid but comparatively similar in way like that rhamnolipid. The pH range was 6.7 to 7 almost neutral whereas the time taken was to be the larger from 72 hours to 120 hours. The agitation it required was 200 rpm. Table 4.

Optimization Techniques

Response Surface Methodology (RSM)

RSM is a statistical technique used to optimize multiple variables simultaneously. By analyzing the effects of factors like carbon source concentrations, pH, and temperature on biosurfactant production, researchers can determine the optimal conditions for maximum yield.[83].

Box-Behnken Design (BBD)

BBD is a type of RSM that helps in exploring the relationships between multiple variables and response variables. This approach has been successful in increasing biosurfactant production by optimizing culture conditions and substrate concentrations.

PROPERTIES OF BIOSURFACTANTS

Biosurfactants are surface active compounds produced by a variety of microorganisms that have potential uses in various industries. A significant factor for their potential use lies in their surface active properties in comparison to chemical surfactants.[84].

Table 4. Biosurfactant production and their operating conditions.

Biosurfactant	Medium and Supplement	Operating conditions				References
		Temperature(C)	pH	Time (hr)	Agitation speed (rpm)	
Rhamnolipid	Mineral Salt medium, Basal Salt medium	25-37	7-7.5	48-288	120-330	[74,76,78,82]
Sophorolipid	Basal salt medium	27-30	5.6-6	72-168	150-200	[73,79,80,81]
MELs	Broth	30	7	216	200	[75]
Surfactin	Mineral salt medium	30-37.5	6.7-7	72-120	200	[72]

Critical Micelle Concentration

The physiology of cell surface and nature of biosurfactant production means that they are more effective at lower concentrations than surfactants and have both a higher or lower critical micelle concentration. This varies widely depending upon the biosurfactant, however many are effective surfactants at concentrations of 10mg/L or less, with the most effective being able to reduce surface and interfacial tensions by more than 70 dyne/cm and 1 mN/m respectively.[85].

This can have beneficial environmental implications, since a reduction in surface tension reduces the likelihood of emulsification and is toxic to aquatic life. A lower critical micelle concentration means that biosurfactants are soluble at lower concentrations than surfactants with higher CMC's and can be microbial carbon sources.

CMC with respect to surface tension reduction of wastewater from 72 to 32 dynes/cm with the CMC value at 30 mg/L as compared with SDS (40 dynes/cm) but at a much higher CMC value of 2000 mg/L [86] Figure 3.

Surface Tension Reduction

The ability to reduce surface tension is still a primary yardstick for surfactant effectiveness, and many chemical surfactants have been developed merely by empirical testing to find more effective compounds. Static and dynamic surface tension measuring methods have been developed to assess the effectiveness of surfactants.

Due to the desirable environmental effects of microbial surfactants, particularly in the case of rhamnolipids produced by *Pseudomonas sp*, it is becoming increasingly common for these testing methods to be used on new microbial surfactants. The surfactant properties of many microbial compounds have even been discovered by noting the reduction in surface and interfacial tension of liquid cultures, with no prior intention to produce biosurfactants[87].

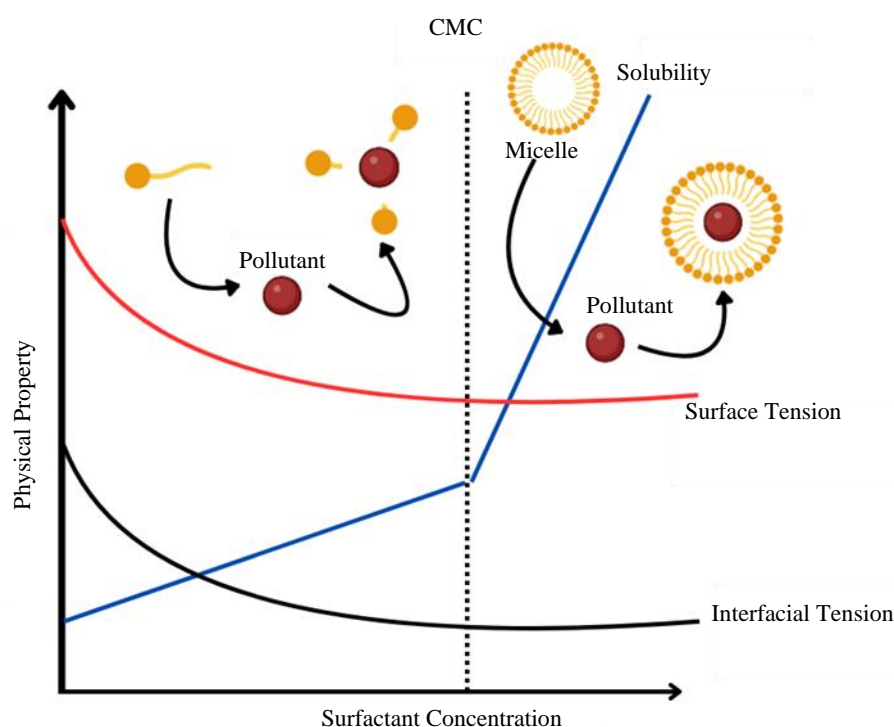


Figure 3. Biosurfactant Concentration and dependence of properties.

Emulsification Ability

The emulsification ability is crucial for biosurfactants as it plays a significant role in various applications, particularly in industries like oil recovery and environmental remediation. Biosurfactants, such as those produced by *Bacillus subtilis* SL, have the capacity to emulsify two immiscible liquids, like hydrocarbons, enhancing the dispersion and bioavailability of hydrophobic pollutants for microbial access [88].

Emulsification mediated by biosurfactants can improve the mobility of crude oil in reservoirs, promoting its extraction and increasing flow rates by mobilizing hydrophobic molecules bound on solid substrates.

The emulsification index and emulsification activity are key screening tests to measure the emulsification capacity of biosurfactants, demonstrating their ability to create stable emulsions and enhance the accessibility of hydrophobic substrates for microbial cells.

This property is essential for various bioremediation technologies and industrial processes, highlighting the importance of emulsification in the effectiveness of biosurfactants. Table 5.

MECHANISMS OF BIOSURFACTANT ACTION

Biosurfactants are classified as special amphiphilic metabolites produced by a variety of microorganisms. They are compounds featuring both hydrophobic (e.g. fatty acids, fatty alcohols) and hydrophilic (e.g. amino acids, anionic groups) moieties. Their uniqueness lies in their surface activity and their lower CMC compared to chemical surfactants, which makes them candidates for usage in the bioremediation field.[95].

The mechanism of reduction in surface tension is based on Gibbs' adsorption theory. When a liquid is present under a gas or another immiscible liquid, the surface will contract or expand to minimize the area, and all forces acting on it will be balanced. Any additional molecules added to the surface will cause an increase in surface area, and the surface will reach the lowest free energy state when the added concentration is multiplied by the product of the molecular area and the surface tension. The surface tension will then be altered by the difference in Gibbs energy, and it is this alteration which controls the surface excess of the molecules.[96].

There are suggested concepts in explanation of how agents alter surface tension. The model most favored as an explanation of how molecules migrate from bulk to the interphase is by **penetration of a shaped hole by a molecule of suitable size and geometry**. The hole is produced by the failure of the solvent molecules to completely cover the solute molecule, and the difference in packing density of the solvent will create a repulsion on the top of the interphase. The molecule will have a maximum potential energy at the point of moving into the second phase, and if the energy is too high, the molecule will return to the bulk phase. If it can pass through without too much activation energy and the hole collapses, then the molecule will be firmly lodged in the interphase. This process can be likened to ballistic motion and reflection of a particle between two turning plates, and it is apparent that solute molecules will have kinetic energy. If this destabilized state caused by its potential energy is too high, the process will fail.

Using this theory, it is possible to apply the concept of **solubilization**, which is the capacity to increase the concentration of a solute in a particular phase (i.e., solid, liquid, gas) by chemicals known as amphiphiles (having both hydrophobic and hydrophilic properties). As most of the soluble materials in water are polar or ionic and water is the most common medium, it is beneficial to consider hydrophobicity as the main cause for insolubility. Here, the material is not repelled or drawn back but into the non-aqueous phase. High solubilization is essentially the transfer of the maximum solute into the interphase, and the rate of transfer can be rapid or slow.

Table 5. Properties exhibited by various biosurfactants based on substrate and microbial strain.

Biosurfactant Type	Producer Microbial Strain	Source	Surface tension reduction	Critical Micellar Concentration (mg/L)	Interfacial tension (mN/m)	EI 24%	Molecular weight	Ref
Rhamnolipid	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas putida</i> , <i>Burkholderia mallei</i>	Waste cooking oil	50 to 29 dynes/cm	50 mg/L	0.25 mN/m	59	500-650 Da	[49]
		Waste Frying Oil				80		[47]
		Coconut Oil				71.7		[52]
	<i>P. aeruginosa</i> PF2	Contaminated soil	73.2 to 30.5	60		55.6		[60,61]
Sophorolipid	<i>Candida bombicola</i> , <i>Starmerella bombicola</i>		33.5 mN/m	9.5 mg/L	1.8 mN/m	600-706 Da		[89,90]
	<i>C. aquaticum</i>	Fish waste	72 to 27.8			87.6		[57]
	<i>C. spp</i>	Bagasse	72 to 33.9			61.6		[58]
Lipopeptides	<i>Pseudomonas fluorescens</i>		26.5 mN/m	150 mg/L	--			[91]
	<i>B. subtilis</i>	Multi-industrial effluent				2.9 to 3.1		[92]
	<i>B. licheniformis</i>	Sunflower oil and glycerol	72 to 48			62.5		[65]
	<i>B. subtilis</i> LB5a	Cassava wastewater	72 to 25.97	28.33		61 % in gas, 74 % in diesel		[67]
Trehalolipid	<i>Rhodococcus erythropolis</i>		29 mN/m	250 mg/L				[93]
		Sugarcane molasses						[50]
	Various types	Crude Glycerol	72 to 28 mN/m			5 to 26		[94]
		Lactoserum	72 to 38			44 to 69		[94]
	<i>L. paracasei</i>	Vineyard pruning waste	72 to 20.9			1.18		[55]

A dynamic equilibrium will be set up between the solute on the original phase with the concentration at which it is solubilized in the new phase, and it can occur within seconds, as seen with alkanes, or never occur at all. The extent of solubilization is determined by the type and amount of solute, the solvent, and the type and concentration of amphiphile.[97].

Micelle Formation

Micelles are essential structures formed by amphiphilic molecules, including biosurfactants, in aqueous solutions. Understanding the mechanism of micelle formation of biosurfactants is crucial for predicting their behavior and applications in various industries. This article delves into the process of micelle formation by biosurfactants and its significance.

Mechanism of Micelle Formation

Micelle formation occurs when amphiphilic molecules, such as biosurfactants, self-assemble in aqueous solutions. The process involves the aggregation of amphiphilic molecules to form structures known as micelles. Biosurfactants, due to their amphiphilic nature, have a hydrophilic (water-attracting) head and a hydrophobic (water-repelling) tail. When biosurfactant molecules are introduced into an aqueous environment, they orient themselves to minimize exposure of the hydrophobic tails to water, leading to the formation of micelles. Table 6.

The mechanism of micelle formation of biosurfactants involves several key steps

Cell Membrane & Biofilm Disruption

Biosurfactants have the ability to induce pore and ion channel formation in lipid bilayer membranes, leading to the destabilization and disruption of cell membranes. This disruption affects the integrity and permeability of the membranes, ultimately resulting in cell death. The mode of action of biosurfactants includes antibacterial, antifungal, antiviral, and antimycoplasma activities, highlighting their broad-spectrum effects on various microorganisms as well organic pollutants.[98] The mechanism of cell membrane disruption by biosurfactants involves several key steps: Figure 4 Table 7.

Table 6. Key Steps in Micelle Formation of Biosurfactants.

Steps	Description
Adsorption	Biosurfactant molecules adsorb at at the water-air or water-oil interface due to their amphiphilic nature to minimize exposure of hydrophobic tails.
Aggregation	As the concentration of biosurfactant molecules increases, they aggregate to form micelles, with the hydrophobic tails shielded from the surrounding water.
Micelle Growth	Continued addition of biosurfactant molecules leads to the growth of micelles, stabilizing the structure and reducing surface tension.

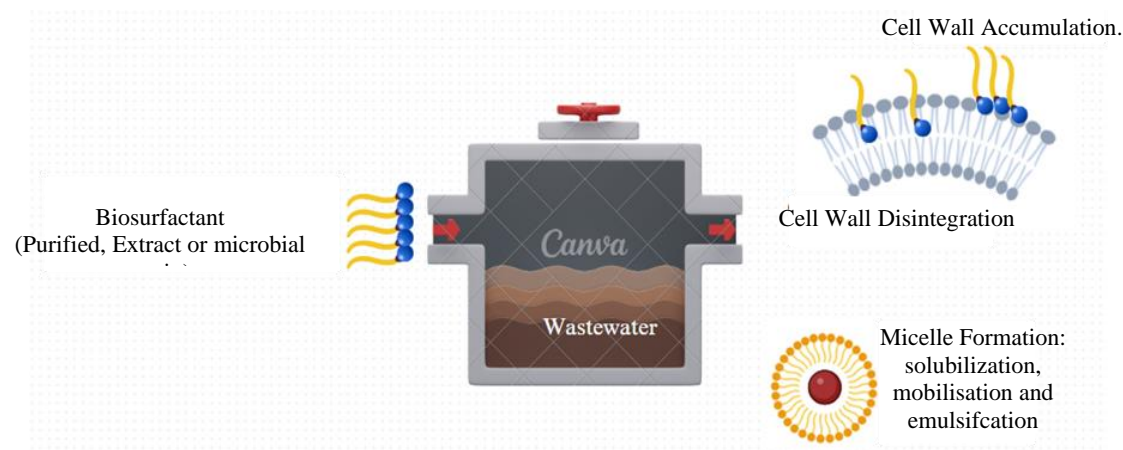


Figure 4. Mechanism of Biosurfactant action in wastewater treatment.

Table 7. Key Steps in Cell Membrane & Biofilm Disruption by Biosurfactants.

Step	Description
Pore and Ion Channel Formation	Biosurfactants induce the formation of pores and ion channels in lipid bilayer membranes.
Destabilization of Membranes	Disruption of cell membranes, leading to destabilization of their integrity and permeability.
Interference with Surface Proteins	Inhibition of the transport of binding surface proteins by biosurfactants.

APPLICATIONS OF BIOSURFACTANTS IN WASTEWATER TREATMENT

Biosurfactants enhance wastewater treatment by promoting the breakdown of pollutants and improving the efficiency of bioremediation processes, offering an eco-friendly alternative to synthetic chemicals.

Table 8. Biosurfactants and their usage in wastewater treatment.

Substrate	Mechanism used for contacting	Biosurfactant used	Removal efficiency and other comments	Ref
Oil removal				
Oily wastewater	Dissolved air flotation and bio surfactant integrated system	<i>Pseudomonas cepacian</i> CCT6659, <i>Bacillus cereus</i> UCP1615	DAF efficiency increases up to 98.55%	[77,99]
	Dissolved air flotation and bio surfactant integrated system	<i>Pseudomonas aeruginosa</i> UCP 0992.	Stability of biosurfactant was constant for different temperatures, exposure time, pH values and salinity. Oil separation efficiency increases to 95%	[100]
		<i>Bacillus methylotrophicus</i> UCP1616. 0.2 wt % potassium sorbate as stabiliser	100% Emulsification activity at all pH values after 3 months. Reduction to 95% after 1 month and less than 50% after 70 days. Oil removal efficiency of 92%.	[101]
	Demulsification	Bacteria Halomonas venusta strain N3-2A	92.5% demulsification of oil within 24 h.	[102,107]
Hydrocarbon degradation				
	Electroremediation	<i>Pseudomonas aeruginosa</i> SR17	86.1% TPH removal	[103]
		<i>Bacillus subtilis</i> AS2	88% crude oil removal	[104]
PAH removal				
Sludge from coking wastewater treatment system	Biodegradation	<i>Pseudomonas aeruginosa</i> S5	43.1% increase of PAHs bioavailability	[76,105]
Wastewater		<i>Pseudomonas aeruginosa</i> UG22	Hexachloroiphenyl, Phenanthrene, Toluene, Ethylbenzene, Butylbenzene, Phenyldecane, naphthalene, pristane, Pyrene, fluoroanthrene removal	[51,76,92]
Wastewater		Rhamnolipid	65 % Bisphenol A removed, Biosurfactant concentration at 1 ppm in 50 ppm contaminant at 25 C, 5.8 pH for 120 minutes	[64,65]
Heavy metal removal				

Wastewater sludge containing heavy metals	Decontamination	Candida tropicalis, Glutamic acid	Cu (65%), Zn (57%), Cr (49%), Pb (47%), Ni (60%) and Mn (70%) removal	[59,106]
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Opportunities and Limitations Environmental Impact

Exploring the balance between potential gains and challenges in addressing environmental impacts.

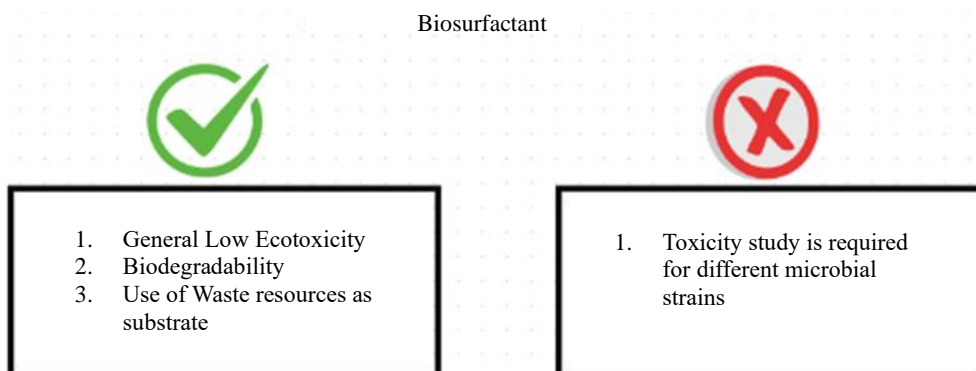


Figure5. Positive and negative Environmental Impacts of Biosurfactants.

Table 9. Cost Analysis of Biosurfactant production rates.

Biosurfactant Origin	Supplier	Cost (£/L)
Bacterial rhamnolipid	BioFuture Ltd., Dublin	0.02
Citrasolv	Cleveland Biotech Ltd., Teesside	0.01
EC601 Bacterial rhamnolipid	Ecochem Ltd., Canada	0.23
EC1800 Bacterial consortium	Ecochem Ltd., Canada	0.01
Petrosolv Bacterial unknown	Enzyme Technologies Inc., USA	0.01
Saponin Plant bark	Sigma UK	0.50

Cost of Production

The cost of production is a crucial factor in determining the economic feasibility and commercial viability of biosurfactants. Understanding the cost analysis of various biosurfactants provides valuable insights into their affordability and potential applications. This article presents a tabular comparison of the cost of production for different biosurfactants based on the provided sources. Figure 5 Table 9.

FUTURE PERSPECTIVES

The key benefits of biosurfactants usage in waste water treatment is for bioremediation as it enhances the biodegradation where it facilitates the breakdown of organic pollutant in waste water and thus make it more biodegradable and environmental friendly the second advantage is the foaming ability which aids in removal of contaminants and pollutants from base water and thus improving the treatment efficiency also biosurfactants gives us a degree of freedom in choosing what type of bio surfactant we can apply for certain waste water treatment they exhibits specific activity against various pollutants and thus make them targeted and effective in waste water treatment application

Enhanced Biodegradation

Biosurfactants facilitate the breakdown of organic pollutants in wastewater, enhancing biodegradation processes.

Foaming Ability

The excellent foaming ability of biosurfactants aids in the removal of contaminants and pollutants from wastewater, improving treatment efficiency.

Specific Activity

Biosurfactants exhibit specific activity against various pollutants, making them targeted and effective in wastewater treatment applications.

CONCLUSION

The usage of bio surfactant is going to be very prominent in coming days as it can be integrated with already existing technologies light for instance enhanced oil recovery or our tertiary is restrict comment also the petrochemical waste sludge treatment and other biological treatments.

Enhanced Oil Recovery

Biosurfactants can be utilized in microbial enhanced oil recovery processes, showcasing their potential in treating oily wastewater streams.

Sludge Treatment

The application of biosurfactants in sludge treatment processes can improve dewatering efficiency and reduce the environmental impact of sludge disposal.

Anti-Biofilm Properties

Biosurfactants possess anti-biofilm properties, making them valuable in combating biofilm formation in wastewater treatment systems, thereby enhancing overall treatment performance.

The use of bio surfactant as waste water treatment has a key commercialization aspects especially with the enhanced treatment necessary for remediation of various type of waste tenths increasing the waste water treatment efficiency which is the need of the hour.

Biosurfactants can improve the removal of contaminants and pollutants from wastewater due to their excellent foaming ability and surface-active properties

Biodegradability

The biodegradable nature of biosurfactants makes them a sustainable choice for wastewater treatment, aligning with environmental regulations and sustainability goals.

Targeted Applications

Biosurfactants exhibit specific activity against various pollutants, allowing for targeted and effective treatment in wastewater applications.

REFERENCE

1. Miao, Y., To, M. H., Siddiqui, M. A., Wang, H., Lodens, S., Chopra, S. S., ... & Lin, C. S. K. (2024). Sustainable biosurfactant production from secondary feedstock—recent advances, process optimization and perspectives. *Frontiers in Chemistry, 12*.
2. Jiménez-Peñalver, P., Koh, A., Gross, R., Gea, T., & Font, X. (2020). Biosurfactants from waste: structures and interfacial properties of sophorolipids produced from a residual oil cake. *Journal of Surfactants and Detergents, 23*(2), 481-486.
3. Rosen, M. J., & Kunjappu, J. T. (2012). *Surfactants and interfacial phenomena*. John Wiley & Sons.
4. Water, U. N. (2017). 2017 UN World Water Development Report: Wastewater the Untapped Resource.
5. Yadav, V. K., Choudhary, N., Ali, D., Gnanamoorthy, G., Inwati, G. K., Almarzoug, M. H., ... & Solanki, M. B. (2021). Experimental and computational approaches for the structural study of novel

- Ca-rich zeolites from incense stick ash and their application for wastewater treatment. *Adsorption Science & Technology*, 2021, 1-12.
6. Yadav, V.K.; Ali, D.; Khan, S.H.; Gnanamoorthy, G.; Choudhary, N.; Yadav, K.K.; Thai, V.N.; Hussain, S.A.; Manhrdas, S. Synthesis and characterization of amorphous iron oxide nanoparticles by the sonochemical method and their application for the remediation of heavy metals from wastewater. *Nanomaterials* 2020, 10, 1551.
 7. Yadav, V.K.; Yadav, K.K.; Gacem, A.; Gnanamoorthy, G.; Ali, I.H.; Khan, S.H.; Jeon, B.-H.; Kamyab, H.; Inwati, G.K.; Choudhary, N. A novel approach for the synthesis of vaterite and calcite from incense sticks ash waste and their potential for remediation of dyes from aqueous solution. *Sustain. Chem. Pharm.* 2022, 29, 100756.
 8. Irannajad, M.; Kamran Haghghi, H. Removal of heavy metals from polluted solutions by zeolitic adsorbents: A review. *Environ. Process.* 2021, 8, 7–35.
 9. Modi, S.; Inwati, G.K.; Gacem, A.; Saquib Abullais, S.; Prajapati, R.; Yadav, V.K.; Syed, R.; Alqahtani, M.S.; Yadav, K.K.; Islam, S.; et al. Nanostructured Antibiotics and Their Emerging Medicinal Applications: An Overview of Nanoantibiotics. *Antibiotics* 2022, 11, 708.
 10. Rahman, L.; Wong, Z.; Sarjadi, M.; Soloi, S.; Arshad, S.; Bidin, K.; Musta, B. Heavy metals removal from electroplating wastewater by waste fiber-based poly(Amidoxime) ligand. *Water* 2021, 13, 1260.
 11. Su, H.; Lin, J.; Chen, H.; Wang, Q. Production of a novel slow-release coal fly ash microbial fertilizer for restoration of mine vegetation. *Waste Manag.* 2021, 124, 185–194.
 12. Cao, M.; Hu, A.; Gad, M.; Adyari, B.; Qin, D.; Zhang, L.; Sun, Q.; Yu, C.-P. Domestic wastewater causes nitrate pollution in an agricultural watershed, China. *Sci. Total Environ.* 2022, 823, 153680.
 13. Yadav, V.K.; Khan, S.H.; Choudhary, N.; Tirth, V.; Kumar, P.; Ravi, R.K.; Modi, S.; Khayal, A.; Shah, M.P.; Sharma, P.; et al. Nanobioremediation: A sustainable approach towards the degradation of sodium dodecyl sulfate in the environment and simulated conditions. *J. Basic Microbiol.* 2022, 62, 348–360.
 14. Madhav, S.; Ahamad, A.; Singh, A.K.; Kushawaha, J.; Chauhan, J.S.; Sharma, S.; Singh, P. Water pollutants: Sources and impact on the environment and human health. In *Sensors in Water Pollutants Monitoring: Role of Material*; Pooja, D., Kumar, P., Singh, P., Patil, S., Eds.; Springer: Singapore, 2020; pp. 43–62.
 15. Bilotta, G.S.; Brazier, R.E. Understanding the influence of suspended solids on water quality and aquatic biota. *Water Res.* 2008, 42, 2849–2861.
 16. Sharma, R.; Agrawal, P.R.; Kumar, R.; Gupta, G.; Ittishree. Chapter 4—Current scenario of heavy metal contamination in water. In *Contamination of Water*; Ahamad, A., Siddiqui, S.I., Singh, P., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 49–64.
 17. Rakib, M.J.; Rahman, A.; Onyena, A.P.; Kumar, R.; Sarker, A.; Hossain, M.B.; Islam, A.R.M.T.; Islam, S.; Rahman, M.; Jolly, Y.N.; et al. A comprehensive review of heavy metal pollution in the coastal areas of Bangladesh: Abundance, bioaccumulation, health implications, and challenges. *Environ. Sci. Pollut. Res.* 2022, 1–27.
 18. Hüesker, F.; Lepenies, R. Why does pesticide pollution in water persist? *Environ. Sci. Policy* 2022, 128, 185–193.
 19. Boulkheissaim, S.; Gacem, A.; Khan, S.H.; Amari, A.; Yadav, V.K.; Harharah, H.N.; Elkhaleefa, A.M.; Yadav, K.K.; Rather, S.-U.; Ahn, H.-J.; et al. Emerging trends in the remediation of persistent organic pollutants using nanomaterials and related processes: A review. *Nanomaterials* 2022, 12, 2148.
 20. Sharma, B. Microbes as indicators of water quality and bioremediation of polluted waters: A novel approach. In *Microbial Biotechnology in Environmental Monitoring and Cleanup*; Pankaj, Sharma, A., Eds.; IGI Global: Hershey, PA, USA, 2018; pp. 44–60.
 21. The United Nations World Water Development Report 2023: partnerships and cooperation for water, 2023 ISBN 978-92-3-100576-3
 22. Dias, M. A. M.; Nitschke, M. Bacterial-derived surfactants: an update on general aspects and forthcoming applications. *Braz. J. Microbiol.* 2023, 54, 103–123.

23. Kirtil, E.; Oztop, M. H. Mechanism of adsorption for design of role-specific polymeric surfactants. *Chem. Pap.* 2023
24. Wokosin, K. A.; Schell, E. L.; Faust, J. A. Emerging investigator series: surfactants, films, and coatings on atmospheric aerosol particles: a review. *Environ. Sci. Atmos.* 2022, 2 (5), 775–828.
25. Tato, J. V.; Seijas, J. A.; Vázquez-Tato, M. P.; Mejjide, F.; deFrutos, S.; Jover, A.; Fraga, F.; Soto, V. H. Introduction to Biosurfactants. *Biosurfactants for a Sustainable Future 2021*, 1–42.
26. Nagtode, V. S., Cardoza, C., Yasin, H. K. A., Mali, S. N., Tambe, S. M., Roy, P., ... & Pratap, A. P. (2023). Green surfactants (biosurfactants): a petroleum-free substitute for Sustainability—Comparison, applications, market, and future prospects. *ACS omega*, 8(13), 11674–11699.
27. Li, H., Fang, C., Liu, X., Bao, K., Li, Y., & Bao, M. (2023). Quantitative analysis of biosurfactants in water samples by a modified oil spreading technique. *RSC advances*, 13(15), 9933–9944
28. Intasit, R.; Soontorngun, N. Enhanced palm oil-derived sophorolipid production from yeast to generate biodegradable plastic precursors. *Ind. Crops Prod.* 2023, 192, 116091.
29. Xu, R.-Q.; Ma, L.; Chen, T.; Zhang, W.-X.; Chang, K.; Wang, J. Sophorolipid inhibits histamine-induced itch by decreasing PLC/IP3R signaling pathway activation and modulating TRPV1 activity. *Research Square*, January 5, 2023
30. Zhang, X.; Wang, Y.; Lu, J.; Liu, M.; Tan, W.; Cheng, Y.; Tao, Y.; Du, J.; Wang, H. Biosurfactant promoted enzymatic saccharification of alkali-pretreated reed straw. *Bioresour. Technol.* 2023, 372, 128665.
31. Nascimento, M. F.; Keković, P.; Ribeiro, I. A. C.; Faria, N. T.; Ferreira, F. C. Novel Organic Solvent Nanofiltration Approaches for Microbial Biosurfactants Downstream Processing. *Membranes* 2023, 13 (1), 81.
32. Matosinhos, R. D.; Cesca, K.; Carciofi, B. A. M.; de Oliveira, D.; de Andrade, C. J. Mannosylerythritol lipids as green pesticides and plant biostimulants. *J. Sci. Food Agric.* 2023, 103 (1), 37–47.
33. Yang, Q.; Shen, L.; Yu, F.; Zhao, M.; Jin, M.; Deng, S.; Long, X. Enhanced fermentation of biosurfactant mannosylerythritol lipids on the pilot scale under efficient foam control with addition of soybean oil. *Food Bioprod. Process.* 2023, 138, 60–69.
34. Mawani, J. S.; Mali, S. N.; Pratap, A. P. Formulation and evaluation of antidandruff shampoo using mannosylerythritol lipid (MEL) as a bio-surfactant. *Tenside Surfactants Deterg.* 2023, 60 (1), 44–53.
35. de Oliveira Schmidt, V. K.; de Vasconcelos, G. M. D.; Vicente, R.; de Souza Carvalho, J.; Della-Flora, I. K.; Degang, L.; de Oliveira, D.; de Andrade, C. J. Cassava wastewater valorization for the production of biosurfactants: surfactin, rhamnolipids, and mannosylerythritol lipids. *World J. Microbiol. Biotechnol.* 2023, 39 (2), 65.
36. Khalafi, H.; Ahmadi, S. A comparative study of Mannosylerythritol lipids (MELs) surfactant adsorption upon the A112N12 and B12N12 nano-cages as potential candidates for detecting MELs. *J. Indian Chem. Soc.* 2023, 100 (1), 100805.
37. Selva Filho, A. A. P.; Converti, A.; Soares da Silva, R. d. C. F.; Sarubbo, L. A. Biosurfactants as Multifunctional Remediation Agents of Environmental Pollutants Generated by the Petroleum Industry. *Energies* 2023, 16 (3), 1209.
38. Johnson, P., Trybala, A., Starov, V., & Pinfield, V. J. (2021). Effect of synthetic surfactants on the environment and the potential for substitution by biosurfactants. *Advances in colloid and interface science*, 288, 102340.
39. Pal, S., Chatterjee, N., Das, A. K., McClements, D. J., & Dhar, P. (2023). Sophorolipids: A comprehensive review on properties and applications. *Advances in Colloid and Interface Science*, 313, 102856.
40. Zhen, C., Ge, X. F., Lu, Y. T., & Liu, W. Z. (2023). Chemical structure, properties and potential applications of surfactin, as well as advanced strategies for improving its microbial production. *AIMS microbiology*, 9(2), 195.

41. Ma, Z., Zuo, P., Sheng, J., Liu, Q., Qin, X., & Ke, C. (2023). Characterization and production of a biosurfactant viscosin from *Pseudomonas* sp. HN11 and its application on enhanced oil recovery during oily sludge cleaning. *Applied Biochemistry and Biotechnology*, 1-17.
42. Soberón-Chávez, G., Lépine, F., & Déziel, E. (2005). Production of rhamnolipids by *Pseudomonas aeruginosa*. *Applied microbiology and biotechnology*, 68, 718-725.
43. Twigg, M. S., Baccile, N., Banat, I. M., Déziel, E., Marchant, R., Roelants, S., & Van Bogaert, I. N. (2021). Microbial biosurfactant research: time to improve the rigour in the reporting of synthesis, functional characterization and process development. *Microbial Biotechnology*, 14(1), 147-170.
44. Gürkök, S., & Özdal, M. (2021). Microbial biosurfactants: properties, types, and production. *Anatolian Journal of Biology*, 2(2), 7-12.
45. Guo, P., Xu, W., Tang, S., Cao, B., Wei, D., Zhang, M., ... & Li, W. (2022). Isolation and characterization of a biosurfactant producing strain *Planococcus* sp. XW-1 from the cold marine environment. *International journal of environmental research and public health*, 19(2), 782.
46. Voulgaridou, G. P., Mantso, T., Anastopoulos, I., Klavaris, A., Katzasta, C., Kiouisi, D. E., ... & Pappa, A. (2021). Toxicity profiling of biosurfactants produced by novel marine bacterial strains. *International Journal of Molecular Sciences*, 22(5), 2383.
47. M. Ozdal, S. Gurkok, and O. G. Ozdal, "Optimization of rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and chicken feather peptone," *3 Biotech*, vol. 7, no. 2, pp. 1–8, 2017
48. E. J. Gudiña, E. C. Fernandes, A. I. Rodrigues, J. A. Teixeira, and L. R. Rodrigues, "Biosurfactant production by *Bacillus subtilis* using corn steep liquor as culture medium," *Front. Microbiol.*, vol. 6, no. FEB, pp. 1–8, 2015
49. G. Yañez-Ocampo, G. Somoza-Coutiño, C. Blanco-González, and A. Wong-Villarreal, "Utilization of ozda waste for biosurfactant production by native bacteria from chiapas," *Open Agric.*, vol. 2, no. 1, pp. 341–349, 2017,
50. T. H. Mouafo, A. Mbawala, and R. Ndjouenkeu, "Effect of different carbon sources on biosurfactants' production by three strains of *Lactobacillus* spp.," *Biomed Res. Int.*, vol.2018
51. G. O. Oyetibo et al., "Biodegradation of crude oil and phenanthrene by heavy metal resistant *Bacillus subtilis* isolated from a multi-polluted industrial wastewater creek," *Int. Biodeterior. Biodegrad.*, vol. 120, pp. 143–151, 2017
52. S. George and K. Jayachandran, "Production and characterization of rhamnolipid biosurfactant from waste frying coconut oil using a novel *Pseudomonas aeruginosa* D," *J. Appl. Microbiol.*, vol. 114, no. 2, pp. 373–383, 2013
53. I. E. Affandi, N. H. Suratman, S. Abdullah, W. A. Ahmad, and Z. A. Zakaria, "Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria," *Int. Biodeterior. Biodegrad.*, vol. 95, no. PA, pp. 33–40, 2014,
54. T. Ndlovu, S. Khan, and W. Khan, "Distribution and diversity of biosurfactant-producing bacteria in a wastewater treatment plant," *Environ. Sci. Pollut. Res.*, vol. 23, no. 10, pp. 9993–10004, 2016
55. X. Vecino, L. Rodríguez-López, E. J. Gudiña, J. M. Cruz, A. B. Moldes, and L. R. Rodrigues, "Vineyard pruning waste as an alternative carbon source to produce novel biosurfactants by *Lactobacillus paracasei*," *J. Ind. Eng. Chem.*, vol. 55, pp. 40–49, 2017,
56. I. M. Banat, S. K. Satpute, S. S. Cameotra, R. Patil, and N. V. Nyayanit, "Cost effective technologies and renewable substrates for biosurfactants' production," *Front. Microbiol.*, vol. 5, no. DEC, pp. 1–19, 2014
57. S. B. Patil, Y. A. Patil Sawant, L. H. Kamble, and C. J. Raorane, "Primary Screening of Actinomycetes in prospects with Biosurfactant Production from Animal fat waste," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 5, no. 2, pp. 92–97, 2016,
58. P. C. Martins and V. G. Martins, "Biosurfactant production from industrial wastes with potential remove of insoluble paint," *Int. Biodeterior. Biodegrad.*, vol. 127, no. November 2017, pp. 10–16, 2018,

59. J. Guo, X. J. Jia, L. Yang, G. Zhang, and L. Zhang, "Effect of biosurfactant on ammonia removal from anaerobically digested swine wastewater by multi soil layering treatment bioreactors," *Environ. Technol. (United Kingdom)*, vol. 41, no. 19, pp. 2510–2517, Aug. 2020,.
60. S. Pourfadakari et al., "Remediation of PAHs contaminated soil using a sequence of soil washing with biosurfactant produced by *Pseudomonas aeruginosa* strain PF2 and electrokinetic oxidation of desorbed solution, effect of electrode modification with Fe₃O₄ nanoparticles," *J. Hazard. Mater.*, vol. 379, no. February, p. 120839, 2019,
61. S. He et al., "Simultaneous degradation of n-hexane and production of biosurfactants by *Pseudomonas* sp. strain NEE2 isolated from oil-contaminated soils," *Chemosphere*, vol. 242, 2020,
62. H. M. M. Ibrahim, "Characterization of biosurfactants produced by novel strains of *Ochrobactrum anthropi* HM-1 and *Citrobacter freundii* HM-2 from used engine oilcontaminated soil," *Egypt. J. Pet.*, vol. 27, no. 1, pp. 21–29, 2018,
63. S. George and K. Jayachandran, "Production and characterization of rhamnolipid biosurfactant from waste frying coconut oil using a novel *Pseudomonas aeruginosa* D," *J. Appl. Microbiol.*, vol. 114, no. 2, pp. 373–383, 2013
64. M. Alshabib and S. A. Onaizi, "Enzymatic Remediation of Bisphenol A from Wastewaters: Effects of Biosurfactant, Anionic, Cationic, Nonionic, and Polymeric Additives," *Water. Air. Soil Pollut.*, vol. 231, no. 8, Aug. 2020,
65. N. A. Jayalatha and C. P. Devatha, "Degradation of Triclosan from Domestic Wastewater by Biosurfactant Produced from *Bacillus licheniformis*," *Mol. Biotechnol.*, vol. 61, no. 9, pp. 674–680, Sep. 2019
66. M. Perez-Ameneiro, X. Vecino, J. M. Cruz, and A. B. Moldes, "Wastewater treatment enhancement by applying a lipopeptide biosurfactant to a lignocellulosic biocomposite," *Carbohydr. Polym.*, vol. 131, pp. 186–196, 2015
67. J. C. Natassia, M. G. Benedito, D. G. Simone, P. R. S. Ana, and M. P. Glauca, "Use of biosurfactant surfactin produced from cassava wastewater for anaerobic treatment of effluent from a poultry slaughterhouse," *African J. Biotechnol.*, vol. 16, no. 5, pp. 224-231, Feb. 2017,
68. Vance-Harrop, M. H., Gusmão, N. B. D., & Campos-Takaki, G. M. D. (2003). New bioemulsifiers produced by *Candida lipolytica* using D-glucose and babassu oil as carbon sources. *Brazilian Journal of Microbiology*, 34, 120-123.
69. Da Rós, P. C., Pereira, T. A., Barbosa, F. G., Marcelino, P. R., & da Silva, S. S. (2024). An Environmentally Friendly Biosurfactant to Enhance the Enzymatic Hydrolysis for Production of Polyunsaturated Fatty Acids with Potential Application as Nutraceutical. *Catalysis Letters*, 154(2), 708-717.
70. Valkenburg, A. D., Ncube, M. Z., Teke, G. M., van Rensburg, E., & Pott, R. W. (2024). A review on the upstream production and downstream purification of mannosylerythritol lipids. *Biotechnology and Bioengineering*, 121(3), 853-876.
71. Ray, S., Sankhyan, S., Sonkar, M., & Kumar, P. (2023). Role of Biosurfactants in the Remediation of Emerging Pollutants. In *Management and Mitigation of Emerging Pollutants* (pp. 411-432). Cham: Springer International Publishing.
72. Liu, Q., Niu, J., Yu, Y., Wang, C., Lu, S., Zhang, S., ... & Peng, B. (2021). Production, characterization and application of biosurfactant produced by *Bacillus licheniformis* L20 for microbial enhanced oil recovery. *Journal of Cleaner Production*, 307, 127193.
73. Shah, V., & Daverey, A. (2021). Effects of sophorolipids augmentation on the plant growth and phytoremediation of heavy metal contaminated soil. *Journal of cleaner production*, 280, 124406.
74. Sharma, S., Verma, R., Dhull, S., Maiti, S. K., & Pandey, L. M. (2022). Biodegradation of waste cooking oil and simultaneous production of rhamnolipid biosurfactant by *Pseudomonas aeruginosa* P7815 in batch and fed-batch bioreactor. *Bioprocess and Biosystems Engineering*, 45(2), 309-319.
75. Machado, T. S., Decesaro, A., Cappellaro, Á. C., Machado, B. S., van Schaik Reginato, K., Reinehr, C. O., ... & Colla, L. M. (2020). Effects of homemade biosurfactant from *Bacillus methylothrophicus*

- on bioremediation efficiency of a clay soil contaminated with diesel oil. *Ecotoxicology and Environmental Safety*, 201, 110798.
76. Cazals, F., Huguenot, D., Crampon, M., Colombano, S., Betelu, S., Galopin, N., ... & Rossano, S. (2020). Production of biosurfactant using the endemic bacterial community of a PAHs contaminated soil, and its potential use for PAHs remobilization. *Science of the total environment*, 709, 136143.
77. Durval, I. J. B., Mendonça, A. H. R., Rocha, I. V., Luna, J. M., Rufino, R. D., Converti, A., & Sarubbo, L. A. (2020). Production, characterization, evaluation and toxicity assessment of a *Bacillus cereus* UCP 1615 biosurfactant for marine oil spills bioremediation. *Marine Pollution Bulletin*, 157, 111357.
78. Venkataraman, S., Rajendran, D. S., Kumar, P. S., Vo, D. V. N., & Vaidyanathan, V. K. (2022). Extraction, purification and applications of biosurfactants based on microbial-derived glycolipids and lipopeptides: a review. *Environmental Chemistry Letters*, 1-22.
79. Wongsirichot, P., Ingham, B., & Winterburn, J. (2021). A review of sophorolipid production from alternative feedstocks for the development of a localized selection strategy. *Journal of Cleaner Production*, 319, 128727.
80. Rodríguez, A., Gea, T., & Font, X. (2021). Sophorolipids production from oil cake by solid-state fermentation. Inventory for economic and environmental assessment. *Frontiers in Chemical Engineering*, 3, 632752.
81. Da Rocha Junior, R. B., Meira, H. M., Almeida, D. G., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2019). Application of a low-cost biosurfactant in heavy metal remediation processes. *Biodegradation*, 30, 215-233.
82. Huang, X., Zhou, H., Ni, Q., Dai, C., Chen, C., Li, Y., & Zhang, C. (2020). Biosurfactant-facilitated biodegradation of hydrophobic organic compounds in hydraulic fracturing flowback wastewater: A dose-effect analysis. *Environmental Technology & Innovation*, 19, 100889.
83. Teja, S. (2021). *A Review on Recent Advances in the Application of Biosurfactants in Wastewater Treatment* (Doctoral dissertation, Faculty of Chemistry, Gdansk University of Technology, Poland).
84. Kumar, P. S., Mohanakrishna, G., Hemavathy, R. V., Rangasamy, G., & Aminabhavi, T. M. (2023). Sustainable production of biosurfactants via valorisation of industrial wastes as alternate feedstocks. *Chemosphere*, 312, 137326.
85. Fung, K. C., Dornelles, H. S., Varesche, M. B., & Gutierrez, T. (2023). From wastewater treatment plants to the oceans: a review on synthetic chemical surfactants (SCSs) and perspectives on marine-safe biosurfactants. *Sustainability*, 15(14), 11436.
86. Sabturani, N., Latif, J., Radiman, S., & Hamzah, A. (2016). Spectroscopic analysis of rhamnolipid produced by produced by *Pseudomonas aeruginosa* UKMP14T. *Malays. J. Anal. Sci*, 20, 31-43.
87. Varnava, C. K., Grenni, P., Mariani, L., Caracciolo, A. B., Hadjipakkou, H., Lefkaritis, G., ... & Tsipa, A. (2024). Characterization, production optimization and ecotoxicity of a lipopeptide biosurfactant by *Pseudomonas citronellolis* using oily wastewater. *Biochemical Engineering Journal*, 205, 109257.
88. Liu, Q., Niu, J., Yu, Y., Wang, C., Lu, S., Zhang, S., ... & Peng, B. (2021). Production, characterization and application of biosurfactant produced by *Bacillus licheniformis* L20 for microbial enhanced oil recovery. *Journal of Cleaner Production*, 307, 127193.
89. Wang, H., Gao, R., Song, X., Yuan, X., Chen, X., & Zhao, Y. (2024). Study on the production of Sophorolipid by *Starmerella bombicola* yeast using fried waste oil fermentation. *Bioscience Reports*, 44(2), BSR20230345.
90. Alara, O. R., Abdurahman, N. H., & Ali, H. A. (2024). Biosurfactant production by utilizing waste products of the food industry. In *Industrial Applications of Biosurfactants and Microorganisms* (pp. 59-77). Academic Press.

91. Sankhyan, S., Kumar, P., Sonkar, M., Pandit, S., Ranjan, N., & Ray, S. (2024). Characterization of biosurfactant produced through co-utilization of substrates by the novel strain *Pseudomonas aeruginosa* NG4. *Biocatalysis and Agricultural Biotechnology*, *55*, 102988.
92. G. O. Oyetibo et al., "Biodegradation of crude oil and phenanthrene by heavy metal resistant *Bacillus subtilis* isolated from a multi-polluted industrial wastewater creek," *Int. Biodeterior. Biodegrad.*, vol. 120, pp. 143–151, 2017,
93. Negrete, P. S., Ghilardi, C., Pineda, L. R., Perez, E., Herrera, M. L., & Borroni, V. (2024). Biosurfactant production by *Rhodococcus* ALDO1 isolated from olive mill wastes. *Biocatalysis and Agricultural Biotechnology*, 103106.
94. A. Wong-Villarreal, L. Reyes-López, C. H. González, C. B. González, and G. Yáñez-Ocampo, "Characterization of bacteria isolation of bacteria from pinyon rhizosphere, producing biosurfactants from agro-industrial waste," *Polish J. Microbiol.*, vol. 65, no. 2 pp. 183–189, 2016
95. Sajadi Bami, M., Raeisi Estabragh, M. A., Ohadi, M., Banat, I. M., & Dehghannoudeh, G. (2022). Biosurfactants aided bioremediation mechanisms: a mini-review. *Soil and Sediment Contamination: An International Journal*, *31*(7), 801-817.
96. Mahapatra, M., Pradhan, S., Preetam, S., & Pradhan, A. K. (2024). Role of Biosurfactants in Heavy Metal Removal and Mineral Flotation. In *Biotechnological Innovations in the Mineral-Metal Industry* (pp. 141-150). Cham: Springer International Publishing.
97. Qi, S., Roser, S., Edler, K. J., Pigliacelli, C., Rogerson, M., Weuts, I., ... & Stokbroekx, S. (2013). Insights into the role of polymer-surfactant complexes in drug solubilisation/stabilisation during drug release from solid dispersions. *Pharmaceutical research*, *30*, 290-302.
98. Dasgupta, A., De, D., & Chaudhuri, S. (2024). Anti-biofilm and disinfectant-like activity of pumilacidin, a lipopeptide biosurfactant produced by *Bacillus pumilus* NITDID1. *Biocatalysis and Agricultural Biotechnology*, *56*, 103024.
99. Etchepare, R.; Oliveira, H.; Azevedo, A.; Rubio, J. Separation of emulsified crude oil in saline water by dissolved air flotation with micro and nanobubbles. *Sep. Purif. Technol.* 2017, *186*, 326–332.
100. Wang, C.; Wang, Z.; Wei, X.; Li, X. A numerical study and flotation experiments of bicyclone column flotation for treating of produced water from ASP flooding. *J. Water Process Eng.* 2019, *32*, 100972.
101. Silva, E.J.; Silva, I.A.; Brasileiro, P.P.; Correa, P.F.; Almeida, D.G.; Rufino, R.D.; Luna, J.M.; Santos, V.A.; Sarubbo, L.A. Treatment of oily effluent using a low-cost biosurfactant in a flotation system. *Biodegradation* 2019, *30*, 335–350
102. Ganesan, N. G., Singh, R. D., Kapila, S., & Rangarajan, V. (2024). The imminent potential of microbial surfactants in PPCP waste removal. In *Development in Wastewater Treatment Research and Processes* (pp. 309-335). Elsevier.
103. Patowary, R., Patowary, K., Kalita, M. C., & Deka, S. (2018). Application of biosurfactant for enhancement of bioremediation process of crude oil contaminated soil. *International Biodeterioration & Biodegradation*, *129*, 50-60.
104. Prakash, A. A., Prabhu, N. S., Rajasekar, A., Parthipan, P., AlSalhi, M. S., Devanesan, S., & Govarthanam, M. (2021). Bio-electrokinetic remediation of crude oil contaminated soil enhanced by bacterial biosurfactant. *Journal of Hazardous Materials*, *405*, 124061.
105. Zang, T., Wu, H., Yan, B., Zhang, Y., & Wei, C. (2021). Enhancement of PAHs biodegradation in biosurfactant/phenol system by increasing the bioavailability of PAHs. *Chemosphere*, *266*, 128941.
106. Tang, J., He, J., Liu, T., Xin, X., & Hu, H. (2017). Removal of heavy metal from sludge by the combined application of a biodegradable biosurfactant and complexing agent in enhanced electrokinetic treatment. *Chemosphere*, *189*, 599-608.
107. Huang, H., Li, Z., Ma, Y., Yao, M., Yao, S., Zhang, Z., & Qin, C. (2023). High-performance arabinoglucuronoxylan-based biosurfactants for oily sludge separation. *Carbohydrate Polymers*, *303*, 120461.