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Preparation, characterization and application of biosurfactant in various industries: A critical review on progress, challenges and perspectives



Teklit Gebregiorgis Ambaye^{a,*}, Mentore Vaccari^a, Shiv Prasad^b, Sami Rtimi^{c,*}

^a Department of Civil, Environmental, Architectural Engineering and Mathematics, University of Brescia, Via Branze

43, 25123 Brescia, Italy

^b Division of Environment Science, ICAR-Indian Agricultural Research Institute New Delhi, 110012, India ^c Global Institute for Water, Environment and Health (GIWEH), 1210 Geneva, Switzerland

Giobal Institute for water, Environment and Health (GiweH), 1210 Geneva, Switzeriana

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ABSTRACT

Due to the increased use of crude oil and other oil-related products, a large amount of waste is produced and discharged into the environment. These wastes contain toxic heavy metals and petroleum hydrocarbon and lead to further deterioration of the terrestrial and aquatic ecosystems. Their increasing amounts and residual leachates are considered the main obstacle to restoring contaminated environments. Biosurfactants are compounds having high emulsification properties, wetting performance, de-emulsification, detergent formulation, foam formation, and surface activity enhancement to minimize the interfacial tension between liquids, a liquid and a gas or a liquid and a solid. Such features make biosurfactants of high potential applications in diverse industrial set-ups. This field attracts attention from scientists (and policymakers) to develop novel, cost-effective and renewable biosurfactants using molecular engineering and emerging downstream processing. This review comprehensively discusses recent applications of biosurfactants, their preparation, characterization, and potential environmental and other industrial applications. The recent advances in biosurfactants using recombinant DNA technology, mutants and hyper-active microbes were also reviewed. We highlighted the use of sophisticated and highly accurate characterization techniques such as high performance-liquid chromatography (HPLC), nuclear magnetic resonance (NMR), thin-layer chromatography (TLC), and gas chromatography-mass spectrometry (GC-MS). Strategies to enhance the efficiency and biosurfactants productivity at a large scale is also discussed.

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Abbreviations: HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; GC-MS, gas chromatography-mass spectrometry; LC-MS, Liquid chromatography-mass spectrometry; NMR, nuclear magnetic resonance; PAH, Polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; DNA, Deoxyribonucleic acid; MALDI-TOF, Matrix-assisted laser desorption/ionization-time of flight mass spectroscopy; HPTLC, High-performance thin-layer chromatography; FT-IR, Fourier Transform-Infrared Spectroscopy; PGR, plant growth-promoting

^k Corresponding authors.

E-mail addresses: t.ambaye@unibs.it (T.G. Ambaye), Rtimi.sami@gmail.com (S. Rtimi).

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1. Introduction

Due to the advancement in technology, people worldwide use different natural resources through exploration and intervention activities. Crude-oil-related products (kerosene, diesel, petrol, crude oil exploration, excavation of fossil fuels), various agricultural, pharmaceutical, and chemical products play a progressive role in the world's current economy. However, some interventions have become detrimental to the environment because of injudicious use of resources like solvents, chemicals, and heavy metals. They also generate unwanted substances or pollutants that can severely impact terrestrial and aquatic environments and affect humans and other creatures (Figueiredo et al., 2019; Wilton et al., 2018). Unwanted poisonous chemicals substances as a pollutant can affect human health by absorbing through human skin or inhalation or accumulate in body parts.

Worldwide, researchers have noticed that these compounds can leach from point and non-point sources to water and soil and indirectly affect human beings by accumulating fish, fruits, vegetables, and other food products, which enhance toxicity, severe injuries, and leads to death (Ahamed and Lichtfouse, 2021). Hence, developing sustainable or low-cost alternative pathways to restore contaminated environments has attracted more attention from policymakers and scientists (Collivignarelli et al., 2018; Jimoh and Lin, 2019a). Different biological techniques such as biodegradation and bioremediation are used to remove pollutants from the environment through mineralization or biochemical solubilization. However, such a process has some limitations, such as affinity to strong soil particles binding, low biological availability, low water solubility, and most of them are expensive. Moreover, these methods are not widely applied to restore the environment due to their cost effectiveness (Bezza and Chirwa, 2015; Chaprão et al., 2015; Ahamed and Lichtfouse, 2021).

As a natural greener material, biosurfactants are presently gaining significant importance because they are ecofriendly, like biodegradation and bioremediation techniques. In addition to this, when compared to chemical surfactants, biosurfactants have many potential advantages such as high selectivity, biodegradability, biocompatibility, bioavailability, and ecological acceptability, increased effectiveness in the extreme condition of temperature and salt concentrations. Currently, tremendous research has been carried out in biosurfactant development and their use to restore contaminated environments and remediation of inorganic and organic contaminants of different sectors. Biosurfactants are successfully applied in microbial enhanced oil recovery, pharmaceutical products, cosmetics, wastewater treatment, and sludge treatment. Furthermore, they are preferably used as an emerging remediation technology for heavy metals removal from water and soil (Bezerra et al., 2018; Sun et al., 2019). However, recently biosurfactant efficiency for biotechnological and environmental applications is limited due to its low insolubility and bioavailability, strong adsorption to soil particles, and pollutant hydrophobicity. Moreover, compared with chemical surfactants, it still has some problems related to the recovery and downstream process and low production yield, making the production cost of biosurfactants expensive (Al-Wahaibi et al., 2014; Jimoh and Lin, 2019b). Therefore, novel biosurfactants must be developed to solve the problems mentioned above and consider their properties, such as improving fermentation and recovery process using genetic engineering, which can apply in different biotechnological and environmental applications.

This paper aims to review the progress and challenges of biosurfactant production using cheap raw material with descriptions of its properties, progress in the improvement of microbial strains, optimization of media components, modified statistical techniques, and the growth of hyper-producing genetic micro-organisms. It also discusses the novel and green techniques for biosurfactants' production using recombinant DNA technology and its application, challenge, and perspectives in different biotechnological industries for environmental sustainability.

Table	1
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Use of chemical substances and their impacts	Use	f chemical	lse of	substances	and	their	impacts.	
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Chemical substances	Examples	Possible impacts	References
Hydrocarbons	Polycyclic aromatic hydrocarbons (PAHs), polyaromatic, unsaturated, saturated, polychlorinated biphenyl (PCBs)	Hydrocarbons have diverse effects on the environment and human health due to their hazardous nature	Kuppusamy et al. (2017)
Heavy metals	Nickel, thallium, cadmium, copper, titanium, zinc, iron, arsenic, mercury	Heavy metals causing severe toxicity to humans and other living organisms, even at very low concentrations	Liang et al. (2017)
Air contaminants	Acid rain, ozone, carbon monoxide, particulate matter, volatile organics (chloroform, xylenes, ethylbenzene, benzene, toluene)	Respiratory diseases. Cardiovascular damage, fatigue, headaches, and anxiety	Posada-Baquero et al. (2019) and Prasad et al. 2021
Organophosphorus compounds	Perchloroethylene, chlorinated, hydrocarbons, nitroaromatic compounds, and trichloroethylene	Organophosphorus compounds have diverse effects on the environment and human health	Lászlová et al. (2018)

2. Use of the chemical substances and their impacts

Chemicals are part of our everyday life. Every living and dead matter is made up of chemicals, and essentially every created product involves chemicals. When properly used, many chemicals can significantly contribute to the quality of life, health, and well-being (Zhang et al., 2012; Liang et al., 2017). Chemical substances used as surfactants are generally employed as emulsifiers or as surface energy reducers, especially at the interface oil–water. A surfactant is considered suitable or not for a particular application based on its solubility, reduction capability, micelle concentration, and wettability. Chemical surfactants are generally petrochemical or oleochemical derivatives and have been applied for large scale applications.

In general, chemicals can be utilized as a raw material in big and small-scale industrial and manufacturing activities, food and consumer product production and storage, and attempts to manage or eradicate insect-borne diseases (e.g., Lyme disease, West Nile virus...). Furthermore, application of chemicals to increase crop yields and control fungi, weeds, insects, and other pests has increased the use of chemical surfactants. Other applications led to surfactants accumulation in surface water such as the improper disposal of household products e.g., lawn care materials, pharmaceuticals, cleaning products, batteries, paint, automotive products... Some chemicals are very hazardous and can negatively affect the health of living beings and the environment when improperly managed (Wu et al., 2017) as summarized in Table 1. These potential contaminants can be transported to sea, lakes, rivers, and soil, which enhance biomagnification and bioaccumulation in creatures over a long period and further affect the aquatic life and terrestrial environment (Kuppusamy et al., 2017; Lászlová et al., 2018; Posada-Baquero et al., 2019).

Hence the effect of the chemicals on the human and environment leads to a quest for the development of technology that aid in the cleanup of inorganic and organic contaminants such as metals and hydrocarbons. The use of biosurfactants and biosurfactant-producing microorganisms is an alternative and environmentally benign way of environmental remediation technology for these pollutants. Biosurfactants are a versatile class of chemicals with potential uses in a wide range of industrial and biotechnological fields (Pacwa-Płociniczak et al., 2011) mainly produced by bacteria, fungi and yeasts.

3. Global market of biosurfactant

Currently, biosurfactants' demand increases globally due to promising biodegradable properties, less toxic, and a green solution of personal care, home care, and environmentally friendly industries. According to the world biosurfactant market estimates, in 2017, its worth value was about 4.20 billion dollars and predicted to grow about 5.52 billion dollars in 2022 with a compound annual growth rate of 5.6%. Global biosurfactants demand/share in various industries are presented in Fig. 1.

The annual growth rate shows a massive demand and application of biosurfactants in different industries around the globe. Among the various biosurfactants, the market for glycolipids is expected to grow at a higher compound annual growth rate due to its high purity and rhamnolipids functionality. Biosurfactants are currently used globally in personal care, pharmaceutical, agriculture, food processing, and detergent manufacturing. Among these applications, biosurfactant demand is highest in detergent production because of its high degradability and removal of hydrophobic strains, and high performance under neutral conditions. In terms of regional demand, the global market of biosurfactants is extended and prevailing from Middle East Africa, North, South, and Central America, Asia Pacific, and Europe to Asia. According to the biosurfactants market report 2016, it was the highest marketed in Europe and forecasted to continue in the future. The regional/continental biosurfactants demand is presented in Fig. 2.

In Europe, the biosurfactants' market increased due to society's high awareness, as they consume more biosurfactants to prevent environmental hazards from chemicals. However, biosurfactants' production has a higher cost mainly because

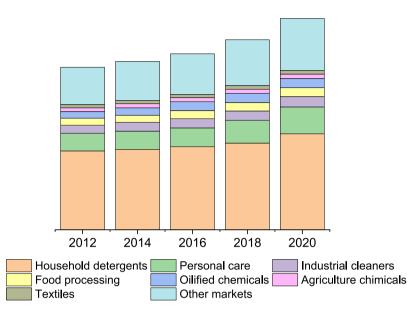


Fig. 1. Global biosurfactants demand and share in various industries Biosurfactants adapted from Market Size, Share & Trends Analysis Report by Product (Rhamnolipids, MES, APG, Sorbitan Esters, Sucrose Esters), By Application (Household Detergents, Personal Care, Industrial Cleaners) And Segment Forecasts, 2014–2020.

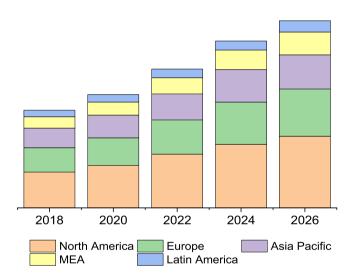


Fig. 2. Regional/continental biosurfactants demand Biosurfactant Market – Global Industry Analysis and Forecast (2019–2026) by Type, application, and geography.

of its low productivity, high raw material cost, and inadequate technology for product purification compared to chemical biosurfactant production. Therefore, biosurfactants production costs should be reduced through the advancement of biosurfactant process technologies.

4. Biosurfactants and their production

Biosurfactants are surface-active molecules and have amphiphilic ends, mediating the surface interactions at the interface through the two ends (Mnif et al., 2014; Mesbaiah et al., 2016). The first end is non-polar groups such as (fatty acids and saturated or unsaturated hydrocarbon chains), having the water-repelling property. The second groups are polar ends such as (mono-, di- or polysaccharides, acid, peptide, anions, or cations), which has the property of water affinity produced by a specific microorganism (Hassanshahian, 2014; Sharma and Saharan, 2016). In the current scenario, biosurfactants as an eco-friendly processed material can be applied in various industries such as oil refinery, food, cosmetics, agriculture, pharmaceutics, and restoring the contaminated environment (Anjum et al., 2016; Chebbi et al., 2020).

Table 2

Microbial strains and	specific use	of biosurfactants.
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Genera	Microbial strains	Substrate used	Biosurfactants yield and specific use	References
	P. cepacia CCT6659	Canola waste frying oil	40.5 g L ⁻¹	(Soares et al., 2018)
Pseudomonas	P. aeruginosa DN1	Palm oil; C/N ratio 20	25.98 g L ⁻¹	Ma et al. (2016)
	P. aeruginosa M408	Olive oil (40 g l^{-1})	12.6 g L^{-1}	Ji et al. (2016)
Rhodococcus	R. erythropolis ATCC 4277	Glycerol, NaNO ₃ , MgSO ₄	0.285 g L^{-1}	Pacheco et al. (2010)
D = -!!!	B. subtilis HSO121	Maltose, L-arginine	$47.58 \text{ g } \text{L}^{-1}$	Haddad et al. (2014)
Bacillus	Bacillus subtilis E8	Soluble starch (80 g l^{-1}),	12.20 g L^{-1}	Gong et al. (2009)
	L. delbrueckii N2	Molasses	2.43 to 3.03 g L^{-1}	Mouafo et al. (2018)
Lactobacillus	L. cellobiosus TM1	Glycerol	2.32 to 2.82 g L^{-1}	Mouafo et al. (2018)
	L. plantarum G88	MRS broth	0.30 to 0.51 \pm g L ⁻¹	Mouafo et al. (2018)
Candida	C. bombicola	Corn liquor, molasses, waste oil 5% (v/v)	61 g L ⁻¹	Pinto et al. (2018)
	C. lipolytica UCP 0988	Animal fat (5%), corn steep liquor (2 ·5%)	10 to 40 g L^{-1}	Santos et al. (2017)
	C. tropicalis UCP0996	Molasses, corn liquor, waste oil (2 ·5%)	7.36 g L^{-1}	Almeida et al. (2017)

Biosurfactants are amphiphilic compounds that can be produced with a range of surface and chemical properties by a varied group of micro-organisms (Li et al., 2016; Martins and Martins, 2018; López-Prieto et al., 2019). The microorganisms used in the production of biosurfactants are ranging from low molecular weight to high molecular weight and belong to different genera such as *Clostridium, Brevibacterium, Pseudomonas, Rhodococcus, Acinetobacter, Thiobacillus, Bacillus, Leuconostoc, Lactobacillus, Enterobacter, Saccharomyces, Aspergillus, Ustilago, Penicillium, Corynebacterium, Citrobacter, Candida and Paenibacillus sp. D9* (Fooladi et al., 2016; Magalhães et al., 2018; Singh et al., 2019; Ahamed and Lichtfouse, 2021). Microbial strains and their specific uses as biosurfactants belonging to various genera are given in Table 2.

Biosurfactants are classified into different groups based on various compound structures such as lipopeptides (surfactin, polymyxin, iturin subtilisin, vixcosin, serrawetin); glycolipids (sophorolipids, xylolipid, mannosylerythritol, cellobiose, lipids, rhamnolipids, trehalose lipids), flavolipid, phospholipids, fatty acids, polymeric surfactants (liposan, alasan, emulsan) lipids and polysaccharide–protein complexes, (Hemlata et al., 2015; Martins and Martins, 2018; Jimoh and Lin, 2019b; Ahamed and Lichtfouse, 2021). The other group of molecules that can be used can replace the biosurfactants referred to as bioemulsifiers. These compounds have an active surface and an emulsion between hydrocarbons and water mixture and quickly decrease surface tension (Gudiña et al., 2015a). However, in some cases, biosurfactants are also referred to as bioemulsifiers if the bioemulsifiers compounds are produced from high molecular weight of heteropolysaccharides, lipoproteins, and lipopolysaccharides (Perfumo et al., 2018; De Souza Monteiro et al., 2012; Smyth et al., 2010).

4.1. Strategy to improve production of biosurfactant

Biosurfactants are natural surface-active compounds that can be produced biologically from microbial strains by using various substrates ranging from hydrophobic mixtures, hydrocarbons, hydrophobic mixtures, solvents, chemicals, waste products, oil wastes, dairy products, and vegetable oils (Gudiña et al., 2015b; Chirwa et al., 2017). The promising strategies for enhanced biosurfactant production and low-cost materials may be microbial strain-engineering, cost-effective carbon sources, improved fermentation process, and downstream processing of the products at the industrial level higher cost-effectiveness (Dell'Anno et al., 2018; Jimoh and Lin, 2019b).

In the past few years, researchers have reported the biosurfactants production from industrial wastes such as liquor, animal fat, soap stock, starch waste, molasses, corn steep, including different agricultural products like vegetable oils, corn, cassava flour, sugar cane bagasse, wheat straw, rice straw, beet molasses, rice bran, etc., as potential substrates for the production of biosurfactants (Darvishi et al., 2011; Datta et al., 2018; Jimoh and Lin, 2019b). However, fermentative production of biosurfactants can be affected by various factors such as temperature, inoculum size, agitation speed, aeration, and stress (Joshi et al., 2008; Chebbi et al., 2018; Jimoh and Lin, 2019c).

The availability and non-availability of nutrients such as phosphorus, manganese, sulfur, iron, nitrogen, and carbon and their ratio, especially C:N, C:Fe, C:P, and C, affect the biosurfactants fermentative processes (Maass et al., 2016; Noha et al., 2018). Therefore, it is imperative to optimized these parameters to enhance the production of biosurfactants for obtaining cost-effective products so that they can be applied effectively in industry at a large scale (Kanna et al., 2014; Lee et al., 2018). Furthermore, to produce biosurfactants economically, it must integrate the production through downstream processing and explore the alternative to improve its production by using the innovative statistical approach of surface methodology, as shown in Fig. 3, along with using genetically engineered bacteria superlative mutants.

As mentioned below, genetically engineered microbial strains, cost-effective substrate, optimized media, improved fermentation process, and downstream processing, purification of end products using well developed statically models can be commercially viable biological and engineering solutions to achieve cost-effective large scale industrial biosurfactants production for the substantiality of the environment (Sekhon et al., 2012; Varadharajan and Subramaniyan, 2014; Sidkey et al., 2016; Jimoh and Lin, 2019a; Jimoh et al., 2021). The schematic representation of various strategies involved in biosurfactant production is shown in Fig. 4.

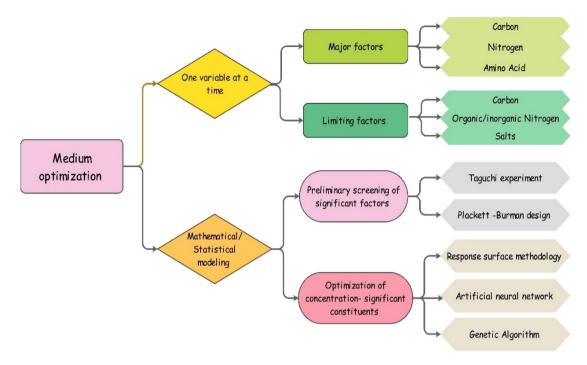


Fig. 3. Graphical representation of various methodologies used in medium optimization.

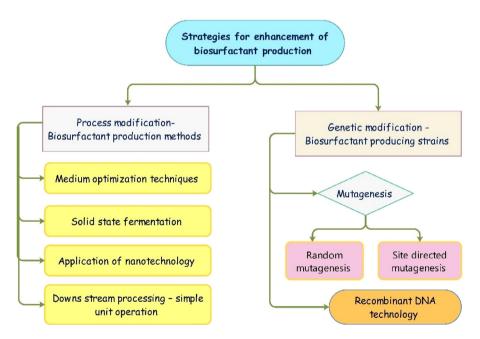


Fig. 4. Schematic representation of various strategies involved in biosurfactants production.

4.2. Production of biosurfactants using recombinant DNA technology

Currently, the biotechnological application using genetic engineering recombinant DNA technology to produce biosurfactants is gaining importance in the scientific community due to the potential of recombinant strains, as they have many utilities from the industrial viewpoint (Hu et al., 2019; Wu et al., 2019). It is well understood that during the production of biosurfactants, the micro-organism can also have the capacity to degrade the different substrates. However, little information about the microbes is used to produce the biosurfactants in their cloning, functional characterization, degradative, and molecular characteristics (Satpute et al., 2016). Furthermore, Sekhon et al. (2012) reported that introducing some specific genes in the presence of carbon and hydrocarbon as a source could increase biosurfactants' production. That has opened a new research area for scientists to develop new microorganisms using recombinant DNA technology to improve biosurfactants' production and efficiency in diverse industrial applications.

According to Bachmann et al. (2014) and De Almeida et al. (2019), microbial strain improvement through recombinant DNA technology can provide higher biosurfactants production yield at a lower cost due to modifying the chemical properties. Moreover, they found that the biosurfactants produced from this process technology can also resist extreme high temperature, salt, and pH conditions. Therefore, if recombinant DNA technology has cloned microbial strain to achieve maximum potentiality to make biosurfactants at the industrial scale, these conditions need to be optimized. There is also a need to introspect the genetic composition characteristics of micro-organisms that limit the biosurfactant's yield (Satpute et al., 2010).

Kandasamy et al. (2019) compared the production of biosurfactants from olive oil using recombinant *Escherichia coli* pSKA clones containing the BioS gene, srfA, and showed that production of biosurfactants was improved as compared to biosurfactants produce from its parent strain of Bacillus sp. SK320 due to the cloning and expression of modified genes and enzymes activities in *Escherichia coli* recombinants. Sekhon et al. (2012) also reported a similar result for biosurfactant production by comparing microbial cloning mutant gene expression. They found that biosurfactant production was increased two-fold from recombinant strain compared to its parent strain. They also claimed that the recombinant strains could improve the product's characteristics, solve the problems related to reducing protein secreted in the system, and recover and purify biosurfactants. These studies showed microbes' potential to enhance biosurfactants' production using Recombinant DNA technology towards the environment (Balan et al., 2019; Hisham et al., 2019; Kubicki et al., 2019; Williams et al., 2019).

5. Characterization of biosurfactants

In the past years, different range of techniques such as high performance-liquid chromatography (HPLC), thin-layer chromatography (TLC), liquid chromatography–mass spectrometry (LC–MS), gas chromatography–mass spectrometry (GC–MS), nuclear magnetic resonance (NMR) spectrometry, and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF) are familiarized for characterizing and identifying the properties of biosurfactants compounds produced by a different micro-organism (Jimoh and Lin, 2019a)

One of the most extensive techniques to identify and detect an unknown ingredient found in biosurfactants is TLC. This technique took place in a sheet of aluminum foil covered with a thin layer of silica gel as adsorbent material and detects the presence or absence of groups such as carbohydrate groups, lipids, and protein (Silva et al., 2014). For example, according to Ibrahim (2018), in their research to characterize the biosurfactant of produced from Ochrobactrum anthropi HM-1 and Citrobacter freundii HM-2 using TLC shows that it was positive to the lipids when treated using iodine vapors and Molash's reagents, respectively, and this indicates that the existence of glycolipid and glycosyl moiety along with lipid moiety on the nature of these biosurfactants of rhamnolipid. However, this shows the absence of an amino group in the biosurfactant when exposed to the ninhydrin solution.

On the other hand, another sophisticated and precise version with the same TLC approach was High-performance thin-layer chromatography (HPTLC). For example, Al-Wahaibi et al. (2014) reported that biosurfactants produced from Bacillus subtilis, B30, isolated, and characterized products showed that the HPTLC technique was more accurate and precise for data obtained as compared to TLC. Many pieces of research have also reported similar findings using HPTLC techniques for the extraction and separation of different groups of biosurfactant compounds depend upon the polarity of the solvent (Biniarz and Łukaszewicz, 2017; Geissler et al., 2017; Joshi et al., 2016; Moro et al., 2018). HPLC is another method used for the separation extraction, performance, and separation of the biosurfactant samples. This method can characterize each component present in a massive volume mixture within a short period in the cascade process. For instance, Dalili et al. (2015) investigated the HPLC technique to purify and identify cyclic lipopeptide's novel structure from biosurfactants produced from Corynebacterium xerosis NS5 termed as coryxin. However, they confirm that it has low efficacy to determine the separated compounds' purity compared to TLC.

Fourier Transform-Infrared Spectroscopy was used to identify the chemicals and organic substances found in the biosurfactants. This spectroscopy technique is used to determine the biosurfactants' molecular components and structures by measuring the sample material's wavelength with infrared radiation (Ibrahim, 2018). For instance, Chakraborty and Das (2017) analyzed the organic chemical constituent using the FT-IR analytical technique. They have shown that all analytes of biosurfactants can be found in the range of 4000 to 400 cm⁻¹, and peak bands could be characterized as O–H stretching and H-bonds consisting of alcohols and phenols, and water. The characterization of the biosurfactant of glycolipid produced from a strain of *Pseudomonas aeruginosa* HAK01 using FTIR spectroscopy showed that the purified biosurfactant presents a peak at 3336 cm⁻¹ which was attributed to the –OH functional group of H bonding. This indicates that the presence of polysaccharides, hydrocarbon chains of C–H bands (CH₂–CH₃) with strong bonds at 2856 and 2924 cm⁻¹ as well as the presence of ester compounds indicated at the stretching peak of 1645 cm⁻¹. In addition to this, the presence of amino acids observed at the bands 1550, 3420, and 3245 cm⁻¹, and these all results indicate that biosurfactant has a long chain of hydrocarbons with rhamnose rings and rhamnolipid structure. FTIR analytical technique, as reported by many researchers, can easily and quickly determine the chemical structure of the biosurfactants at a low cost (Chooklin et al., 2014; Datta et al., 2018; Patowary et al., 2018).

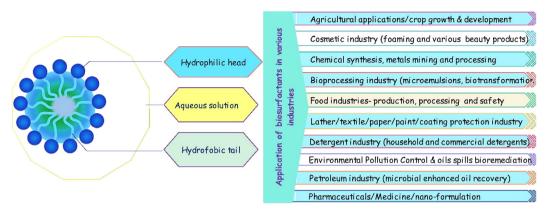


Fig. 5. Application of biosurfactants in various industries.

Mass spectrometry is also used for determining the structure and chemical bonds present in the biosurfactant sample. In this method, the instrument is coupled with liquid or gas chromatography to identify its structure and gives the qualitative and quantitative analysis of each compound present in the biosurfactants sample (Sharma et al., 2014). The same result was also reported by Ibrahim et al. (2013), who showed that the hydrophilic and hydrophobic parts of the biosurfactants produced from bacteria could be analyzed by using LC–MS and GC–MS respectively. It has more advantages with accuracy than HPLC in the molecular determinants of the compound. In addition to the above methods, mass spectrometry coupled with electrospray ionization to identify the different biological molecules such as proteins, peptides from biosurfactants using high voltage separate fragments of macromolecules of ions produced gives better and precise accuracy (Monteiro et al., 2007). Jimoh and Lin (2019b) investigated that electrospray ionization coupled with liquid chromatography helps to identify the biomolecule with low concentration and secondary metabolites as in the biosurfactant compound. For instance, Yin et al. (2009) also used electrospray ionization coupled with liquid chromatography to identify the chemicals constituents from biosurfactants produced from *P. aeruginosa S6*, including four crucial amino acids, such as *RhaC12:1C10*, *RhaC8C10*, *RhaRhaC10C12:1*, and *RhaC10C10*.

Likewise, Guo et al. (2012) used matrix-assisted laser desorption/ionization-time of flight mass spectroscopy to identify the chemical constituent found in biosurfactants made from the genus *Paenibacillus*. They found that the biosurfactants have 13 amino acid residues with cyclic lipopeptide and finally noted that using this coupled technique can help identify the different complex compounds with less time with high-resolution data for the essential characterization in lass cost. However, it demands more energy for the formation of ions. NMR technique is also used widely by researchers for identifying chemical structures found in biosurfactants. This spectroscopic technique determines the biosurfactants' structure and establishes its purity and composition (Chakraborty and Das, 2017). Li et al. (2016) used the NMR technique to characterize the constituent found in the biosurfactants produced from *Bacillus pseudomycoides BS6*, having a long chain of long hydrocarbon in the chemical shift of the 0.8–1.4 and 3.3–5.5 ppm regions as well as the presence of sugar moiety in the structure of the biosurfactant indicated at the signals at of 0.49 and 4.87 ppm. Moreover, they claimed that this technique could determine deuterated solvents used to digest biosurfactants before going to characterization and further contended that it needs more study to optimize the solvent to identify each component of the biosurfactants quickly.

6. Application of biosurfactants

As discussed above, biosurfactants have unique properties due to their cell surface's hydrophobicity. These hydrophilic and hydrophobic properties of biosurfactants decreasing the surface tension between block hydrogen and immiscible or miscible liquids. In addition to this, due to their substrate specificity, rapid, controlled inactivation and degradation properties, biosurfactants can be used in remediation technology in hydrocarbon and microbial enhanced oil recovery, food, commercial laundry detergent, paint industries, petrochemicals, medicine, textiles, pollution control as well as mediated biosynthesis of metallic nanoparticles (Geetha et al., 2018; Kandasamy et al., 2019; Olasanmi and Thring, 2018; Yuliani et al., 2018). Various applications of biosurfactants in industrial product manufacturing are shown in Fig. 5.

Examples of biosurfactants application in various industries and households, especially petroleum oil recovery enhancer, bioremediation/cleaning oils spills, precious metals mining, agriculture, and food processing industries, medicine/ pharmaceuticals/bioprocessing, lather/textile/paper/paint/coating protection, chemical synthesis, cosmetics, and detergents production are presented in Table 3.

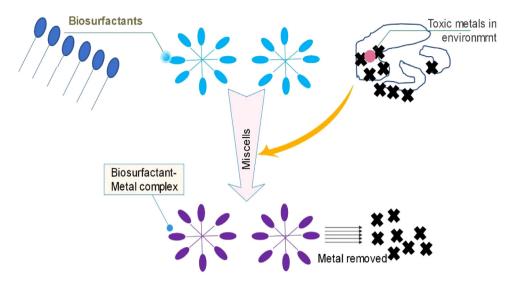


Fig. 6. The removal of potentially toxic elements through the mediation of biosurfactants. Adapted from (Fenibo et al., 2019).

6.1. Application of biosurfactants for restoring contaminated environments

Worldwide rapidly increasing economy and industrialization have boosted the use of chemical surfactants, leading negatively due to many toxic chemicals discharged to the environment. Around the world, a large amount of wastewater is released to the water bodies and pollutes the surface and drinking water. Organic and inorganic pollutants generated from hazardous waste and its dumping in the environment have a negative impact on human health and severe effects on the ecological system and dangerous to the environment (Makkar et al., 2011; Meenakshisundaram and Pramila, 2017; Olasanmi and Thring, 2018). Removing such pollutants from the environment is the biggest challenge for government, policymakers, scientists, and societies. Therefore, there is a need to develop safer environmental rules and legislation and develop sustainable technologies that remove pollutants from the environment.

In recent years, few researches have been carried out on biosurfactants' utilization to restore the contaminated environment. Biosurfactants are highly biodegradable and non-toxic for creatures and valuable in restoring the polluted environment (Lima et al., 2011). Globally much research has been carried out to compare the effect of biosurfactants with synthetic surfactants on the remediation of pollutants. Many researchers have found that biosurfactants have anionic and cationic properties that decrease the lethal chemical's toxicity in the aquatic and terrestrial environment compared to synthetic surfactants (Vijayakumar and Saravanan, 2015).

The other advantage of biosurfactants is their ability to act as antibiofilm, and an anti-adhesive agent to different contaminates. That leads to microbial biofilm and improves the microbial interaction of the hydrophobicity surface of the active biosurfactants to remove the pollutants from the contaminated environment (Banat et al., 2014). Karlapudi et al. (2020) investigated the properties of various biosurfactants produced from different micro-organisms. They found that biosurfactants produced from micro-organisms possess highly powerful anti-cancer, anti-microbial, and anti-biofilm potential of biosurfactant extracted from an Acinetobacter M6 strain. These unique properties of biosurfactants produced from micro-organisms improve the solubilization and bioavailability of the toxic substances.

The unique properties of biosurfactants were also used to remediate hydrophobic compounds and heavy metals from the contaminated environment. However, when biosurfactants were compared with synthetic surfactants, they have less efficiency in removing heavy metals from contaminated soil. So biosurfactants need further investigation to remove heavy metals from the polluted environment and apply on an extensive scale (Bustamante et al., 2012; Mao et al., 2015).

The application of biosurfactants for removal of heavy metals from the polluted wastewater showed biosurfactants could remove the heavy metals through the complexation process by the attraction of the negatively charged molecules of the biosurfactants with the cation of the heavy metals, as shown in Fig. 6 (Lal et al., 2018; Sarubbo et al., 2018). Therefore, biosurfactants can be utilized for wastewater treatment to improve the environmental and health aspects of society. Biosurfactants as future green remediation technology needs further investigation using different wastewater to study the removal of phosphorus, nitrogen, detergents, pesticides, and hydrocarbon, heavy metal, and other pollutants.

Jimoh and Lin (2019a) investigated the removal of heavy metals using biosurfactants produced from Paenibacillus sp. D9 at different concentrations with and without the formation of aggregates. They showed that the biosurfactants produced from the Paenibacillus sp. D9 has a high removal efficiency of lead and copper and lower efficiency for removing the zinc due to the biosurfactants' weak binding affinity. They also claimed that the concentration of bases or acids, charge of heavy metal, soil properties, and the biomolecule present in the pollutant could influence biosurfactants' affinity to

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Table 3

Examples of biosurfactants application in various industries and households.

Industry	Biosurfactant	Field	Mechanism/Functioning	Reference
Petroleum/enhance oil recovery.	Glycolipids and Lipopeptide	Crude oil extraction from reservoirs	Enhance stable emulsion formation, break down oil film in the rock, reduces tensions and capillary forces that impede oil flow through the rock pores	Almeida et al. (2017)
	Emulsan, alasan, biodispersan	Crude oil pipelines/Transport	Form a stable water-in-oil emulsion that aids oil mobility, viscosity reduction, prevents drop coalescence	Perfumo et al. (2010)
Bioremedia- tion/cleaning oils spills	Glycolipid and Trehalose	Spill remediation	Solubilize oil spills make them available to hydrocarbon-degraders for faster biodegradability	Souza et al. (2014)
	Rhamnolipids Lipopeptides	Treatment of soil and wastewater	Act as emulsifiers/de-emulsifiers, bioavailability enhancers, reduce tensions, mobilize, remove oil/ chemicals from the soil	Ahmad et al. (2018)
	Rhamnolipids, sophorolipids	Hydrocarbon remediation	Solubilize contaminants into the aqueous phase, increase their bioavailability for biodegradation.	Aulwar and Awasthi (2016)
	Rhamnolipids	Heavy metal remediation	Heavy metal remediation from soils by metal entrapment, ion exchange, interactions, binding, desorption, and mobilization.	Aşçı et al. (2010)
Agriculture and Food industry	Glycolipid Rhamnolipids lipopeptides	Soil quality Plant protection	Soil-related toxic metals and other pollutants bioremediation Act on the target cell by disrupting cell surface structures of the plant pathogen	Marchut-Mikolajczyk et al. (2018) Oluwaseun et al. (2017)
	Lipopeptides	Pest control	Detergency property exhibit toxicity against nematodes and insects	Zhao et al. (2010)
	Rhamnolipids	Food stabilizer	Modification of food to a desired consistency and texture	Campos et al. (2013)
Medicine /Pharmaceuticals /bioprocessing	Rhamnolipids	Anti-microbial agent	Act as an anti-microbial agent, manifested through detergent-like activities	Lee and Song (2018)
Josephocessing	Sophorolipids	Anticancer activity	As an antiviral agent, check cell replication in favor of cell differentiation	Yuewen et al. (2017)
	Sophorolipid Rhamnolipids	Antiviral activity Antibiotics recovery	Inactivation of viral lipid envelopes and capsid Extraction of antibiotics and	Muthusamy et al. (2008) Chai et al. (2019)
	Kiannonpius	Antibiotics recovery	proteins using their surfactant properties	chai et al. (2017)
Mining precious metals	Biodispersan	Precious metal recovery	Biosurfactant producing micro-organisms convert (Ag–Au) NO ₃ to silver/gold particles using an enzyme such as nitrate reductase.	Eswari et al. (2018)

(continued on next page)

remove heavy metals and toxic compounds from the contaminated environment. Luna et al. (2016) have investigated adding of hydrochloric acid with biosurfactants produced from *Candida sphaerica*. They showed that the biosurfactants introduced with HCl could remove about 95% of Fe and Zn with a concentration of 0.1% of the biosurfactants.

Biosurfactants can also be used to remove PAHs from the contaminated aquatic environment due to the adsorption and solubilization of the biosurfactants towards the PAHs. Lászlová et al. (2018) reported the biodegradation and adsorption of PCBs and hydrophobic organic compounds from the polluted aquatic environment. They found that biosurfactants' application can increase the pollutants' solubility and enhance the pollutants' bioavailability between the hydrophobic

Table 3 (continued).

Industry	Biosurfactant	Field	Mechanism/Functioning	Reference
Leather/Textile/ Paper/ Paint/coating	Biodispersan	Detergent, emulsifier	Degrease use of detergent, emulsifier; tanning and dyeing agent	Fracchia et al. (2014)
protection	Trehaosete- traester Unspecified cHAL2	Pre-treatment	Remove lipophilic components from fiber surface, oil from fibers, and enhanced dispersion of dyes for uniform and better fiber penetration.	
	Biodispersan	Pulp processing	Washing and deresinification of pulp by defoaming, dispersion	
	Biodispersan	Papermaking	Used as a filter in papermaking, calendaring through the coating, and coloring	
Cosmetic industry	Sophorolipids Rhamno- lipids/MELs	Cosmetics products	Used in cosmetics product manufacturing due to low irritancy, anti-aging agent, antioxidant, moisturizing, healing, and skin toning properties	Roy (2017)
Laundry detergents	Sophorolipids MEL		Used in the detergent making due to foaming agent, surface tension reducer, solubilizer properties	Vecino et al. (2017)

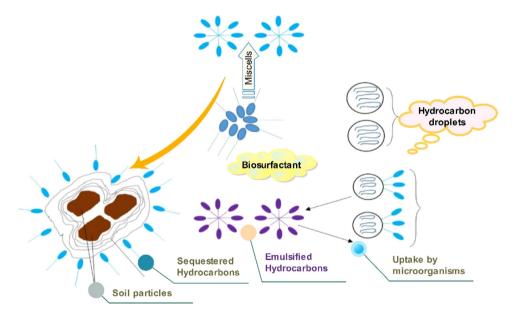


Fig. 7. Hydrocarbon and biosurfactants interaction with soil during the bioremediation process. Adapted from (Fenibo et al., 2019).

and micro-organisms contaminates, as shown in Fig. 7. However, they advised that further consideration is needed to optimize the additives to apply on a large scale as a greener remediation technology.

6.2. Application of biosurfactants in food processing industries as greener alternatives

Biosurfactants also have shown potential applications in the processing industry. It has unique properties, such as emulsion, anti-adhesive, and anti-microbial activities, to benefit food processing. These properties enhance emulsification, surface area with high stabilization of products that can provide safe and healthy food to consumer health on a large scale. Nitschke and Silva (2018) investigated the possibility of using biosurfactants in the food industry at a laboratory scale (Giri et al., 2017). They confirm that biosurfactants' utilization as additives in the food is safe or can remove heavy metals from the products. They also claimed that biosurfactants could also enhance the removal of toxic substances present in food crops, vegetables, and soil. Jimoh and Lin (2020) reported that biosurfactants produced from *Bacillus sp.* MTCC 5877 can

remove Cd from the vegetables by about to 73%, while biosurfactants produced from *Pseudomonas putida* can remove Zn by 50% compared to control. Overall, when compared with synthetic surfactants, biosurfactants have high anti-biofilm and anti-microbial activities related to food pathogens. That makes biosurfactants one of the main additives in food processing.

6.3. Application of biosurfactants in bio-nanotechnology

The integration of biosurfactants produced from microbes with nanoparticles is currently considered the next generation of alternative green chemistry or bio-nanotechnology sources. Biosurfactant-mediated synthesis of nanoparticles has tremendous potential in remediating the contaminated environment (Christopher et al., 2018; Joanna et al., 2018). However, the synthesized biosurfactant-mediated nanoparticles must be cost-effective. In addition, it must have zero-energy demand and high toxicant removal efficiency, and environmental compatibility.

Gómez-Graña et al. (2017) reported that microbes in the biosurfactants could stabilize and reduce nanoparticle formation. Likewise, Rane et al. (2017) showed that nanoparticles such as gold and silver could be produced from microorganisms. This biologically active nanoparticle production opens a new vision for other researchers and scientists to make metallic nanoparticles using a reducing agent of biosurfactants.

Kumar et al. (2010), reported that the biosurfactants could produce from *Brevibacterium casei* MSA19, and it reduces the nanoparticles and allows them to stay stable for about two months by reducing the formation of aggregates through the electrostatic force of attraction and this enhance to serve as an eco-friendly material for different product service. However, the research on stabilizing nanoparticles using biosurfactants is still in the initial stage. So, there is an urgent need to carry out more research to stabilize nanoparticles using biosurfactants before applying them to different nanotechnology applications.

6.4. Application of biosurfactants in the petroleum industry for oil recovery

Biosurfactants can also be used in the petroleum industry to clean up oil spills, remove oil residue from storage tanks, and microbial-enhanced oil recovery. Many investigators reported that as compared to chemical surfactants, biosurfactants are more selective. As a result, they are required in small quantities and more effective under a broader range of oil and reservoir conditions. It can also be used in the various industrial processes to increase solubility, lubrication, mobility, and removing soils and scouring (Karlapudi et al., 2018; Lee and Song, 2018; Liu et al., 2018).

Nerurkar et al. (2012), conducted research using biosurfactant produced from Bacillus licheniformis JF-2 in oil injection water and found that biosurfactant was more effective thermotolerant and anaerobic as compared to other surfactants. In another study, Das (2018) recovered oil from saturated sand oil column using biosurfactants produced from two strains of *Bacillus subtilis* MTCC1427 and MTCC2423. The author showed that biosurfactants can recover oil around 62% in-situ experiments and more stable in the pH range from 4.5 to 10.5.

Jha et al. (2016) reported that biosurfactants could be used for bio-solubilization, biosorption, and bioremediation of pollutants from the environment. They claimed that it could also be used in the petroleum industry to recover microbial enhanced oil, oil immobilization and storage tanks, and oil immobilization due to stable environmental conditions. Similarly, (McClements and Gumus, 2016) also reported that biosurfactants could act as emulsifiers or de-emulsifiers to remove hydrophobic pollutants from the environment. In addition to this, they have also reported that as compared to synthetic surfactants, biosurfactants have high emulsifying activities and enhance ecological compatibility with the environment.

The efficiency of biosurfactants and chemical surfactants for remediation of petroleum pollutants was studied by Satpute et al. (2017, 2018). They showed that the biosurfactants have high efficiency in removing the petroleum pollutant than the chemical surfactants. This may be due to biosurfactants higher surface action and more compatibility with the environment. In addition to this, biosurfactants are less toxic, eco-friendly, and biodegradable, which can be considered greener technology towards the environment (Arora, 2018a,b; Chowdhary and Bhargava, 2019).

6.5. Application of biosurfactants in the pharmaceutical industry

Biosurfactants can also be used in different pharmaceutical and biomedical applications due to their intrinsic antimicrobial properties and the ability to act as anti-adhesive surfaces and have a disruptive biofilm structure. Chen et al. (2017) reported that biosurfactants could be used in various pharmaceutical industry applications. Their intrinsic antimicrobial makes them suitable molecules for fighting various diseases and disorders as therapeutic agents in respiratory failure, antiadhesive, immunological adjuvants, inhibition of pathogens, stimulation of skin fibroblast metabolism, or even in cancer.

Chakraborty and Das (2017), revealed that biosurfactants have high emulsion-forming ability properties compared to chemical surfactants. Therefore, they can be used as anti-microbial agents in the cosmetics and pharmaceutical industries. In addition to this, other researchers confirmed that when compared with synthetically available surfactants, biosurfactants have remarkable properties to produce in large scale commercial formulation of industrial products of cosmetics and drugs as they have high resistance to extreme environmental conditions, make them primary raw material in those industries (Bockmühl, 2012; Rincón-Fontán et al., 2018; Vecino et al., 2017).

Biosurfactants are used in various products formulations such as detergent, ornamentals, cosmetics, pharmaceuticals, antifungal, antibacterial, germicidal, anti-cancer products, wound dressings, skin and personal care products due to their emulsifying, foaming, phase dispersion, emulsion polymerization, and emulsification and de-emulsification property (Bratovcic et al., 2018; Dave and Joshi, 2017; Sil et al., 2017). They are more stable in the extreme condition of temperature range between (50–100 °C), pH (2–12), and even at a high salt concentration (Al-Wahaibi et al., 2014; Bockmühl, 2012; Dhundale et al., 2018). In general, biosurfactants have a huge biotechnological application, but it needs further research advancement to produce stable biosurfactants on a large scale.

6.6. Application of biosurfactants in agriculture and agrochemical industries

To meet the rising population demands for various food products, agricultural production is a matter of big concern for every nation. The application of green technology to achieve sustainable agricultural productivity is a necessity. Biosurfactants can be used in herbicides, fungicides, and pesticides as plant protection measures. It has the potential to enhance the nutrients available for beneficial plant-associated microbes. There are specific reports that infer biosurfactants' role in promoting soil health by soil remediation (Thavasi et al., 2014). Biosurfactants in the industries act as adjuvants with insecticides, herbicides, pesticides, and fungicides, due to their property like emulsifying, spreading, dispersing, and wetting agents' efficiency.

Biosurfactants can be considered a vital part of modern agriculture (Mnif and Ghribi, 2015). Hassen et al. (2018) reported that biosurfactants produced from bacteria of Pseudomonas sp. and Burkholderia sp could use as safe biopesticides. In addition to this, these pesticides can be made from cationic, anionic, anionic, and amphoteric surfactants. Certain biosurfactants from microbes have anti-microbial activity against plant disease. They can be considered a promising biocontrol agent for sustainable agriculture. Biosurfactants facilitate the biocontrol mechanism of plant growthpromoting (PGR) microbes such as parasitism, antibiosis, competition, induced systemic resistance, and hypovirulence. Agricultural pesticides produced from biosurfactants can effectively use in different field crops. It has opened the door to boost agrochemical industries to formulate new chemicals by combining different biosurfactant mixtures and polymers for various agricultural applications.

6.7. Application of biosurfactants in the cosmetic industry

Biosurfactant in the cosmetic industry plays an important role, as an active surface substance in our daily-consume products (Vecino et al., 2017). The unique biosurfactant properties such as de-emulsification, foaming, emulsification, spreading, wetting properties affect the viscosity of product consistency and water-binding capacity, make it a primary utilization material in the cosmetic industry. Biosurfactants are used in making personal care cosmetics products such as skin moisturizers, perfumes, aftershave lotions, lipsticks, eye, and facial makeup preparations, nail polishes, shampoos, hair colors, toothpaste, contact lens solution, acne pads, body massage products, antiperspirants, and denture cleansers, etc. Biosurfactants are used in baby care products, antiseptics, soap, oil, lotions, and creams. Health and beauty products as foot care, lotions, creams, films, gels, sprays, sticks, powders, pastes, and liquids can be prepared or even replaced by biosurfactants (Ferreira et al., 2017; Rincón-Fontán et al., 2018). Monoglyceride is one of the most widely used biosurfactants produced from glycerol tallow via Pseudomonas fluorescens lipase treatment. It can be effective in removing whiteboard marker stains compared to chemical surfactants die due to having high foaming capability of the biosurfactants (Turbekar et al., 2014). Biosurfactants are currently considered an alternative substitute for chemical surfactants due to their higher biodegradability and low toxicity properties.

7. Ecotoxicity study of biosurfactants

Biosurfactants could act as indicators in determining the germination index, which includes developing seeds and roots to assess biosurfactants' toxic effects. In an experiment conducted by Da Rocha Junior et al. (2019), the germination index was found >80%, which shows that the biosurfactants' introduction was non-existence phytotoxicity. Jimoh and Lin (2020) studied the toxic effect of biosurfactants on the cabbage germination index. They introduced it at different concentrations of 1, 10, 100, 200 mg/L and showed a higher value for the germination index: 92.6, 87.8, 89.8, and 94.7%, respectively. That demonstrated that biosurfactants toxicity is dependent upon the concentration of the biosurfactants introduced to the environment. Likewise, research also reported by Bezerra de Souza Sobrinho et al. (2013) in a study to determine the toxic effect of biosurfactant introduced at concentration of 10, 50, 125, 250, 500, and 1000 mg/L on cabbage seeds. In this study, the germination index values were 34, 108, 111, 100, 83, and 81%, respectively. This experiment also explains that increasing the concentration of biosurfactants can inhibit seed germination or root elongation. However, studies on biosurfactants application' aspects of assessing its ecotoxicity on environments need to be investigated systematically.

8. Future perspectives

It is known that biosurfactants have a significant advantage in biotechnological and environmental applications of industrial settings. However, the study on the synthesis, utilization of biosurfactants through different environmental complexes, and limiting factors to a large scale still need more refinement. Therefore, research must focus on producing novel biosurfactants that can rapidly recover product and microbial degradation of pollutants. Research also focused on exploring suitable microbes with high-level metabolic activities through genetic engineering, molecular biology, and surface science, making biosurfactants economically competent to apply in different industries such as cosmetics, pharmaceutics, textile, petroleum, oral hygiene, wastewater treatment, and agriculture. The other important area to be considered in the future is proper understanding the knowledge of the biomolecules and the precise monitoring and testing method to screen the best biosurfactant producers, which are still unknown. Further, research is needed to understate the biosurfactant pathway's biosurfactant pathway at the gene and species level using genomics and proteomics principles.

9. Conclusions

This review showed the widespread prospects of biosurfactants in the nanotechnology product formulation, petroleum industry, personal health care products making, pharmaceutical industry, agriculture, agrochemical industries, food processing industries, including restoring contaminated environments and their sustainability. Biosurfactants are natural, greener, and eco-friendly substitutes for chemical or synthetic surfactants. They can be produced from bioresources by selecting low-cost material and biotechnological approaches at lower production costs. However, its biotechnological and environmental applications process can be inhibited due to its low insolubility and bioavailability, strong adsorption to soil particles, and pollutant hydrophobicity. This issue can be managed by optimized growth/production conditions using economically feasible renewable substrates and efficient multi-step downstream processing. This would help to produce a more profitable biosurfactant. Furthermore, knowing the social and economic benefits of these materials, the optimal conditions for their preparation need to be further investigated.

CRediT authorship contribution statement

Teklit Gebregiorgis Ambaye: Conceptualization, Writing – original draft. **Mentore Vaccari:** Supervision. **Shiv Prasad:** Writing – original draft. **Sami Rtimi:** Conceptualization, Supervision, , Writing – review & editing last version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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