

Production and Application of Biosurfactants in Biotechnology

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Scopus Author ID 36730727200

Received: 27.09.2020; Revised: 20.10.2020; Accepted: 22.10.2020; Published: 26.10.2020

Abstract: Biosurfactants possess both hydrophilic and hydrophobic areas and are generated on the microbial membrane or excreted over the outer membrane. Amphipathicity leads to reduce strength and interfacial tension between the individual molecules on the surface and the two-state immiscible sector. Regarding the low critical micelle concentration (CMC) and high surface activity, biosurfactants can be effective alternatives to their synthetic equal one. Plant-based oils and fats are used in biosurfactants production. Many wastes are produced by the oil and grease industries, tallow, residual oils, marine oils, soapstock, burnt oils, and Manipura. The operation of industrial fatty acid excesses is promising for expansion and transformation. Via making various substances like olive oil mill, acid, whey, and molasses, the agro-industry can ease biosurfactant creation. Biosurfactants have many advantages over chemical production, involving capable of decomposition higher by bacteria or living organisms, less poison, environmental concordance, higher foaming, and the rate of its selection is higher. They can also adjust to the highest salinity, pH, and temperatures and can be produced out of renewable materials, resulting in an increased demand for biosurfactants. Bioemulsifiers and biosurfactants (BSs) have various applications and are a considerable character in many industrial fields, as well as biotechnological features, including pollutant biodegradation, microbial enhanced oil recovery (MEOR), and pharmaceuticals. This review the latest information and improvement in biosurfactant application and development for more output and future applicability.

Keywords: microbial surfactant; CMC; surface tension; oil recovery.

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1. Introduction

Biosurfactants are bio surface-active agents which are produced via numerous microorganisms [1, 2] and highly requested of the market regarding their distinctive eco-friendly characteristic. As the discovery of purified biosurfactant, which has known as a surfactin, they have greatly increase examining some of their features revealed that they are not very desirable in most aspects of human life. At present, so-called synthetic substances that the main ingredients are oil, are most in demand. Most of these compounds are detrimental material and non-decomposable [3]. Since they are involved in bioaccumulation, their performance, yield, and by-products may have serious environmental consequences. Strict

laws were passed to protect the environment; thus, the replacement of chemically produced surfactants with biosurfactants is crucial [4]. In a different subject, biosurfactants are known as high-efficiency molecules. They are widely used in studies of hydrocarbon bioremediation and increase retrieving the oil from the well (EOR) [5]. Universally, 17 MMT surfactants (with soaps) were produced at future pace increases of 3-4% per annum. On the other hand, 1.5-2.0% of various surfactant applications in the EU depend on their usage [6]. In addition, the exclusive feature of biosurfactant leads to efficient use in agriculture 2%, chemical processes 10%, cleaners, and detergents 54%, cosmetics, and pharmaceuticals 3%, food industry 3%, leather and paper 10%, textiles 13%, and other items are 8%, thus gaining a good position. However, most compounds are still being produced during the chemical process from hydrocarbons, albeit surface-active molecules of an organism (i.e., biosurfactants) have gained remarkable attention in recent years. Biosurfactants possess both hydrophilic and hydrophobic areas produced on the microbial membrane or excreted across the outer membrane. Amphipathicity leads to decrease strength and interfacial tension between the individual surface molecules and the two-state separate part (Fig 1) [7, 8]. They have numerous industrial and environmental features concerning emulsification to form foam, detergency and dispersal, wetting, bioremediation, MEOR, and hydrophobic soluble compounds [5].

Biosurfactants are not harmful to the environment and less poison, rendering them decomposable compared to chemically produced surfactants. Additionally, high foaming, carefully choosing something most suitable, and certain potency, an extensive range of capabilities and renewable feedstocks in the production approach are distinctive merits of biosurfactants that could be derived [9]. These compounds reduce interfacially and surface tension in hydrocarbon mixtures and water solutions [10], hence making them a potential possibility for EOR [11] and the emulsification process [12]. Ascribe a high price market to the expansion of biosurfactants [13], complicated procedures of pure substance collection, rare microbial strains (low product concentration capability for those generally found). Biosurfactants have lately attracted much attention due to their high capacity in industrial and environmental operations. Biosurfactants are vital as a consequence of antimicrobials features, biofilm infraction, food industry, healthcare, to eliminate heavy metal, decomposition of hydrocarbon, MEOR, and quorum-sensing (QS). Biosurfactants are excreted by plenty of microorganisms, such as bacteria, fungi, yeast, and actinomycetes exterior of the cell. The majority of them are synthesized by bacteria. Microbial surfactants are indeed lipid molecules whose occurrence, possession, and chemical structure have been extensively investigated in some cases such as antibacterial activities, etc. They are only produced during the growth of microorganisms on hydrophobic substrates [14]. *Bacillus salmalaya*, *Candida lipolytica*, *Pseudomonas aeruginosa*, *Saccharomyces lipolytica*, *Rhodococcus*, and *Corynebacterium* are some microorganisms of this type. Another class of surfactants is both water-soluble and hydrophobic, including *Pseudomonas aeruginosa* and *Torulopsis bombicola*. Hence, the composition and nature of the substrate must be measured to select the appropriate microorganisms [5]. In this review, the latest approaches in knowledge about biosurfactant applications and biotechnological strategies were being examined and expanded for better output of various aspects.

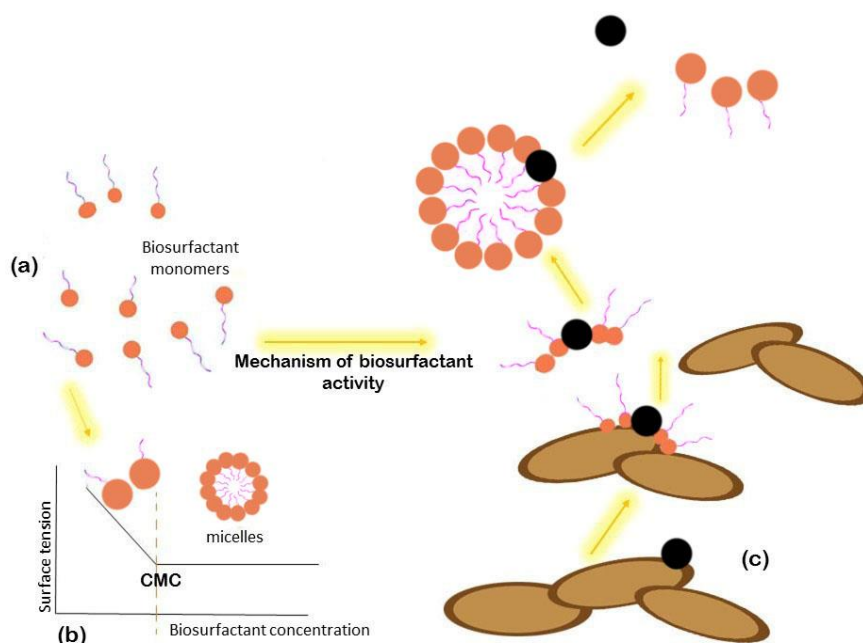


Figure 1. a) Biosurfactant monomers; b) Critical micelle concentration or CMC; c) Mechanism of biosurfactant's activity.

2. Potential commercial applications

All surfactants are synthesized chemically. Nevertheless, ample thought has been newly directed toward biosurfactants. They have been preferred over artificial surfactants attribute to their broad capabilities, the synthetic capacity of biodegradable microbes, and lower toxicity. Some of the industrial properties of biosurfactants have been predicted [15].

2.1. Oil industry.

Multi-biotech, a secondary technology from Geodyn, has commercialized biochemical pharmacology for better use in oil recovery. *Bacillus licheniformis* JF-2, collected from the accumulation of water injected into the oil field, synthesizes most promising biosurfactants (CMC, 10 $\mu\text{g}\cdot\text{l}^{-1}$, the interfacial tension of the salt-decane interface, which decreases to 3-10 dynes cm^{-1}). It has other benefits as well, such as being halotolerant, thermotolerant, and anaerobic, propounding them a potential compound in MEOR [11, 16].

2.2. Heavy metal-binding.

Certain biosurfactants like rhamnolipids are capable of extracting the number of the component from the soil, such as Pb, Zn, and Cd. Rhamnolipids can reduce the poison of metal by using Cd complexation and their interactions with the cell surface to modify Cd uptake. High-molecular-weight (HMW) of polysaccharides in emulsifiers interact with metals through linking them, as noted for uranium connecting by the emulsifier *Acinetobacter calcoaceticus* [17].

2.3. Food industry.

Biosurfactants have numerous thriving qualities in the food industry as a food supplement. Fatty acid esters (FAEs), including sorbitan or ethylene glycol, glycerol, lecithin, and its derivatives, and the producer of ethoxylated monoglycerides (EMGs) involving

manufactured oligopeptides are now used as emulsifiers in the food business around the world [15].

2.4. Cosmetic industry.

Many substances are synthesized for practical cosmetic usage. For illustration, Whole cells and various lipases utilize for the enzymatic transformation of hydrophobic molecules. Biosurfactants, the ones appropriate for cosmetics, should have at least three years to remains usable. Thus, saturated acyl groups are desire over unsaturated monoglycerides. Surfactants are mostly utilized in cosmetics for creating glycerol from tallow (1.5:2) using the lipase from *Pseudomonas fluorescens* (usage percentage = 90%) [18].

2.5. Pharmaceutical industry.

Prematurely neonatal have respiratory issues related to the lung surfactant, which can validate normal using a protein-phospholipid complicated (PPC). This protein can have medicinal properties due to fermentative production by cloning the corresponding genes to bacteria. Tobacco mosaic virus (TMV), mainly from *Nicotiana glutinosa* and potato virus X (PVX) can be treated and controlled by 1% rhamnolipid emulsion [19].

2.6. As emulsifiers.

Biosurfactants can assist the emulsification of water/hydrocarbon mixtures, that this is the section that is well investigated. They have been shown to heighten hydrocarbon decomposition, supply them applicable in managing oil spills [19].

2.7. Peat dewatering.

Surfactin has proved to fetch in mechanical dewatering in a particular volume of space is below 13 g. ton⁻¹ [11].

3. Properties of biosurfactants

Several properties of biosurfactant are lessening surface tension, foaming capacity (FC), emulsifying (EC), stabilizing capacity, reducing CMC, solubility and detergency. These physicochemical features of biosurfactants are crucial in estimating the proficiency and selection of microorganisms that are responsible for the generation of biosurfactants [20]. Despite various chemical constituents and properties, multiple characteristics are common to the majority of biosurfactants, many of which suggest its primacy over ordinary surfactants [13].

3.1. Surface and interfacial activities.

At a diminished mass, biosurfactants yield less surface tension, show greater potency and productivity compared to prosaic surfactants. CMC of biosurfactants (competence measurement) varies from 1 to 2000 mg/l, while surface and interfacial tension (oil/water) are rough 1 and 30 ml/m in the order [21].

3.2. Many biosurfactants can operate in extreme conditions.

Lipopeptides from *Bacillus licheniformis* JF-2 sustain its stability at temperatures around 75°C for 140 h within a pH series of 5 to 12. Biosurfactants also endure salt concentrations of up to 10%, while 2% of salt is adequate to inoperative chemically produced surfactants [21].

3.3. Biodegradability.

Biosurfactants are degradable by bacteria and other microorganisms in water or soil, enable them sufficient for bioremediation and waste treatment purposes [22].

3.4. Low toxicity.

Biosurfactants are now highlighted as a consequence of rising apprehension over the allergenicity of synthetic products [22]. Biosurfactants can also be easily used in cosmetic, pharmaceutical, and food products, an account of their reduction of the poisonous [23].

3.5. Availability.

Biosurfactants are a host of applications as they have been produced from industrial waste and raw compounds [24].

3.6. Specificity.

As complex organic molecules (COMs) with their functional groups, biosurfactants have particular functions, an issue of considerable interest through detoxification of specific contaminants, as well as application in cosmetics, pharmaceuticals, and foodstuff [25].

3.7. Biocompatibility and digestibility.

Biocompatibility and digestibility lead to the employment of biosurfactant in the cosmetic, pharmaceutical, and food industry. On the contrary, Biosurfactants have limitations, too, in a case, mass-produce has a high cost. However, this can dominate when cheap substrates are combined [26]. It is difficult to develop high-purity product extracts since metabolic broth purification requires taking several steps. Highly productive species are scarce and beneficial. Known species in this field cannot produce high surfactant extract and need a complex culture medium. Biosurfactants are biological molecules that probably to be made as a secondary metabolite or in connection with microbial growth; moreover, manipulating the procedure of biosurfactant's procedure is remaining enigmatic. Increasing productivity is often hampered by foam formation, requiring a dilute medium [1].

4. Types of biosurfactants

Chemically synthesized surfactants are organized to establish their polar grouping. In contrast, biosurfactants are principally organized by their microbial source and chemical constituents. Their composition generally consists of a hydrophilic moiety of amino acids or peptide cations or anions, monosaccharides, disaccharides, or polysaccharides, and a hydrophobic compound is embracing saturated, unsaturated, and fatty acids. As a result, the main classes of biosurfactants are proceeding. There are various types of biosurfactants like

Glycolipids, Rhamnolipids, Trehalolipids, Sophorolipids, Lipopeptide, and Lipoprotein, Fatty Acids, Phospholipids, and Neutral Lipids, Polymeric (Table 1) [27].

Table 1. Main classes of biosurfactants and respective producer microorganisms.

Class/type of Biosurfactant	Microorganism	
Glycolipids	Rhamnolipids	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas</i> , <i>Chlororaphis</i> , <i>Serratia</i> , <i>Rubidea</i>
	Sophorolipids	<i>Torulopsis bombicola</i> , <i>T. antarctica</i> , <i>Torulopsis petrophilum</i> <i>C. botistae</i> , <i>C. apicola</i> , <i>C. riodocensis</i> , <i>C. stellata</i> , <i>C. bogoriensis</i>
	Trehalolipids	<i>Rhodococcus erythropolis</i> , <i>Mycobacterium</i> sp. <i>Arthrobactersp.</i> , <i>Nocardia erythropolis</i> , <i>Corynebacterium</i> sp.
	Cellobiose lipids	<i>Ustilagomaydis</i>
	Mannosylerythritol lipids	<i>Candida antarctica</i> , <i>Kurtzmanomyces</i> sp., <i>Pseudozyma Siamensis</i>
	Glycolipid	<i>Streptococcus thermophiles</i>
	Diglycosyldiglycerides	<i>Lactobacillus fermentum</i>
Lipopeptides and lipoproteins	Peptide–lipid	<i>Bacillus licheniformis</i>
	Streptofactin	<i>Streptomyces tendae</i>
	Viscosin	<i>Pseudomonas fluorescens</i> , <i>Leuconostoc mesenteriods</i>
	Serrawettin	<i>Serratia marcescens</i>
	Surfactin	<i>Bacillus subtilis</i>
	Subtilisin	<i>Bacillus subtilis</i>
	Gramicidin	<i>Bacillus brevis</i>
	Polymyxin	<i>Bacillus polymyxa</i>
	Serrawettin	<i>Serratia marcescens</i>
	Polyol lipids	<i>Rhodotorula glutinis</i> , <i>R. graminis</i>
	Ornithinelipids, lysine peptides	<i>Pseudomonas</i> sp., <i>Thiobacillus</i> , <i>thiooxidans</i> , <i>Agrobacterium</i> sp., <i>Streptomyces sioyaensis</i> , <i>Gluconobactercerinus</i>
	Pumilacidin	<i>Bacillus pumilus</i>
	Iturin	<i>Bacillus subtilis</i>
	Sulfonylipids	<i>T. thiooxidans</i> , <i>Corynebacterium alkanolyticum</i>
Lichenysin	<i>Bacillus licheniformis</i>	
Arthrofactin	<i>Arthrobacter</i> sp., <i>Corynebacterium</i> sp.	
Fatty acids, neutral lipids phospholipids	Fatty acid	<i>Corynebacterium lepus</i> , <i>Capnoytophaga</i> sp. <i>Penicillium spiculisporum</i> <i>Arthrobacter paraffineus</i> <i>Talaromycestrachyspermus</i> , <i>Norcardia erythropolis</i>
	Neutral lipids	<i>Nocardia erythropolis</i>
	Phospholipids	<i>Thiobacillus thiooxidans</i>
Polymeric surfactants	Emulsan	<i>Acinetobacter calcoaceticus</i>
	Biodispersan	<i>Acinetobacter calcoaceticus</i>
	Liposan	<i>Candida lipolytica</i> , <i>C. tropicalis</i>
	Carbohydrate–lipid–protein	<i>Pseudomonas fluorescens</i> , <i>Debaryomyces</i> , <i>Polmorphus</i>
	Mannan–lipid–protein	<i>Candida tropicalis</i>
	Protein PA	<i>P. aeruginosa</i>
Particulate surfactant	Vesicles& fimbriae	<i>Acinetobacter calcoaceticus</i> , <i>P. marginilis</i> , <i>P. Maltophilia</i>
	Whole Cells	Various bacteria such as <i>Cyanobacteria</i>
	Alasan	<i>A. radioresistens</i>
	Particulate surfactant (PM)	<i>Pseudomonas marginalis</i>
	Biosur PM	<i>Pseudomonas maltophilia</i>

5. Colloidal properties of some biosurfactants

The CMC (efficiency measurement) varies from 1 to 2000 mg/l, whilst surface tension and interfacial tension (oil/water) are approximately 1 and 30 ml/m, in order. Insufficient expertise about biosurfactants' CMC is the cause to be a challenging issue to interpret or correlate. Table 2 compares the CMC of biosurfactants. The CMCs for the previous group is much lower. A lower CMC originally suggests a more efficient surfactant, cause to be proper for industrial administration [28].

Table 2. Illustrates some examples of the CMC of certain biosurfactants and chemical surfactants.

Surfactant	CMC (mg/L)	Surface tension
Phosphatidylethanolamine	30	25mN/m
Sorborolipid	150	30-35mN/m
Rhamnolipid	20	24mN/m
Surfactin	11	27mN/m
Emulsan	25-58	2-10 mN/m
Sodium lauryl sulfate	8.2	55mN/m

6. Application of biosurfactants in microbial-enhanced oil recovery

Biosurfactants are one of the most promising substances in MEOR. They are employed to recuperate residual oil in the tank after the main (i.e., mechanical) and secondary (i.e., physical) recovery [11, 29]. EOR is a vital third operation during which microorganisms or their metabolites, like acids, biomass, biopolymers, biosurfactants, enzymes and gases, and solvents, are utilized to recuperate secondary oil from drained sediments. The residual oil is often situated in hardly accessible regions of the pool where the oil gets stuck in the pores by linear force. Interfacial tension can be reduced using biosurfactants in oil/rock and oil/water mode, thereby, via the reduction in linear forces, restrict oil from moving via the orifice. To form an emulsion, biosurfactants can also link strongly with the oil/water interface. This fixes the oil desorbed in the water and eliminates it by injecting water in the laboratory, which examines the strategy of recuperation crude oil from a water-saturated column. This involved the required substrate (normally sand), which is utilized to prove the productivity of biostructures in oil recuperate. For further research, the reader is referred to as the surfactant yield. Biosurfactants have other administrations in the oil industry [22]. The demulsifying features of some biosurfactants can be exploited for emulsion breakdown that appears in different phases of petroleum extraction and procedures, allowing greater yield recovery. Distinct oil from the bottom of tanks can be accrued by microbial surfactants that decrease surface tension [30]. Despite some field tests in the literature, yet it is not clear whether microorganisms involved are effective in the oil recuperate operation or whether they contend with autochthonous bacteria [29].

7. Production of biosurfactant

Biosurfactants have been developed by several researchers using a variety of carbon roots and microorganisms [31]. Carbohydrates, vegetable oils, and hydrocarbons are a notable example that is employed as the origin of carbon to synthesize biosurfactants. From a financial point of view, biosurfactants are in a disparity with surfactants [5]. Other sources of carbon, including olive oil wastewater (OOW), whey from the production of cheese and cassava flour, vegetable oils, molasses (by-products) are not appropriate for applying in the industry [32]. Manufacturing process by some bacteria such as *Bacillus* sp. isolates using molasses, whey, and manure as a substrate to produce biosurfactants from *P. aeruginosa* mutant obtained by accidental mutagenesis with N-Methyl-N-nitro-Nitrosoguanidine [32].

7.1. Biosurfactant production by the newly isolated and hopeful strain *Pseudomonas putida* 21BN.

As a kind of biosurfactant, rhamnolipids are generally excreted by *Pseudomonas* sp. that are amphiphilic, active glycolipids compounds on the surface [33].

7.2. Mannosylerythritol lipids [18] produced by *Candida antarctica* as a surface-active.

Lin *et al.* (X) studied lipopeptides produced by *Bacillus licheniformis* mutant by N-methyl-N,N-nitrosoguanidine-mediated accidental mutagenesis [34]. Makkar *et al.* (X) reviewed the creation of lipopeptide biosurfactant by two strains of the genus *Bacillus* with employing molasses in a thermophilic situation [35]. It can be exchanged for microbial enhancement and biosurfactant yield consisting of agro-industrial by-products, peat hydrolysate, and urban disposals [36]. Maugard *et al.* (X) examined the transesterification of fatty acid methyl esters (FAME), and various amines lead to the production of amide surfactant [37]. Kim *et al.* (X) explored biosurfactants creation by *Bacillus subtilis* C9 by applying a carbohydrate (the origin of the carbon). Concurrently a hydrocarbon substrate inhabited the biosurfactant yield, lipopeptide biosurfactant emulsified hydrocarbons, crude oils, and vegetable oils [38]. Osman *et al.* (X) studied Rhamnolipid's products, a multifunctional glycolipid biosurfactant, and pyocyanin, a phenazine produced dye by *Pseudomonas* strain BOP100 from ethanol as a single source of carbon [33]. Ochsner *et al.* (X) checked biosurfactant production induced by *P. aeruginosa*. It is associated with high amounts of biosurfactant of rhamnolipid, which is completely replied to the environmental situation [39]. Biosurfactants are produced by applying OOW as a source of carbon by *Pseudomonas* sp. Nevertheless, peat pressed, urban wastes, and agro-industrial consequences, such as acid whey and OOW, are candidates for biosurfactant yield and microbial growth [40].

7.3. Halophilic bacteria production of biosurfactant.

Halophiles (halophilic or salt-loving microorganisms) are significant regarding their essential features of high salt concentrations for growth. They have matured physiologically and genetically to grow and progress under hypersaline state [41-44]. After Larsen [45] and Kushner [35], due to their salt subservience and tolerance, these organisms have been recognized to be light, medium, and severe halophiles [46]. Most halophiles inhabit hypersaline waters and soils, salt or salt deposits, and salt products [46, 47]. Multi reservoir solar salterns represent general thalassohaline water systems with salinities from seawater salinity to rock salt saturation that are associated with changes in microbial community characteristics [48]. Similarly, aerobic, anaerobic, and facultative anaerobic microbes that belong to Archaea and Bacteria, which are recovered through a thalassohaline waters of the Dead Sea, Salt Lake, the hypersaline lakes in Antarctica, Lake Magadi [49]. Many researchers have reported polysaccharides secreted from haloarchaea and halobacteria, and a constituent of the genus *Halomonas* have been detected as feasible producers [8, 10]. The features of extracellular polysaccharide in halobacteria have been expanded, and their possible administration has been highlighted for the last few years. The principal creator of extracellular polysaccharides (EPSs) reported to date has been recognized by members of the families *Alteromonadaceae* and *Halomonadaceae*. Members of the genus *Halomonas*, the most typical medium-sized halophilic bacteria, have been designated as a feasible creator of EPS that synthesize various polymers of various physical and chemical characteristic [50, 51]. Some of the EPS-producing microorganisms are *Alteromonas hispanica* strain F32T, *Halomonas alkaliantarctica* strain CRSS, *H. alkaliphila*, *H. almeriensis*, *H. anticariensis*, *H. eurihalina* F2-7, *H. eurihalina* A1-12, *H. maura* S-30, and *H. rifensis* [50].

8. Biosurfactant extraction, purification, and characterization

Biosurfactant purification can be mediated via solvent extraction [52]. First, for obtaining cell-free supernatant (CFS), there is a need to centrifuge at 4°C for 20 min. Second, utilize 6N HCl to obtain pH 2.0 and then extract twice with an equal proportion of relaxed ethyl acetate [53]. Subsequently, via the rotavap, transform the liquid to gas shape, therefore absorb the oil which remains yellow and wash with ddH₂O. In the next step, purify and lyophilize, respectively. The biosurfactant which lyophilizes conduct by thin-layer chromatography (TLC) and then remove and apply for the adsorption chromatography on silica gel. The compounds which purified in advance should identify via phase conversion of HPLC with SPD-20A/20AV UV-Vis system (Shimadzu HPLC LC-2010AHT) and C18 column (4.6mm×250mm). The dried KBr pellet sample analyzes by utilizing a Fourier-transformed infrared (FT-IR) spectrophotometer (PerkinElmer 580 B, USA) in a spectrum range of 5000-400 cm⁻¹ using an intuitive software interface Spectrum 10 to collect spectral data. The water (D₂O) analyze in the Bruker JNM-A500 spectrometer (Bruker BioSpin AG, Switzerland) at 400 MHz [53]. Biosurfactants that extracted from CFSs through Folch's extraction method employ for lipid extraction from biomolecules. Folch's extraction method was described in other articles [53].

9. Degradation pathway

Presumably, many bacteria growing on alkanes mediate the increased active effect of PAHs by producing biosurfactants to help resolve PAHs. For instance, the produced biosurfactant by *P. aeruginosa* growing on naphthalene or phenanthrene has been proved to increase the apparent solubility of these PAHs [54]. It appears that natural surfactants increase the deletion of PAHs from the soil by enhancing the PAH solution, thereby raising PAH-microbial interactions [55]. Surfactant-enhanced remediation [39] has been used extensively in PAH-poison soil [56]. Bioremediation persuade by microbial decomposition is often used; natural and affordable materials help as supportive matrices for this method to be promising. Bio-decomposition research has highlighted the storage of isolated bacteria to strengthen the bacterial population, followed by an enhanced in the PAH bio-decomposition product. Nonetheless, there are a few studies on the inoculation-based increased effectiveness of isolated bacteria [57]. Various microorganisms such as algae, bacteria, or fungi have potent to be selected for PAH biodegradation [58-60]. The microorganism-induced PAH biodegradation is subject to a large number of excellent studies [22, 61]. The biodegradation of tricyclic PAH compounds (i.e., low molecular weight (LMW)) has been well studied. LMW PAHs are more degradable than their HMW counterparts [22]. Microbial PAH degradation occurs in three modes: complete mineralization, co-metabolic transformation, and non-specific oxidation [62, 63]. Another form of biodegradation is PAH biodegradation through aerobic microorganisms. For effective disinfection of contaminated areas, it is a good idea to have complete mineralization to prohibit the accumulation of metabolites. The complete mineralization of several PAHs has been identified for some bacterial strains associated with various phylogenetic classes [61].

There are two major PAH degradation mechanisms, reliant upon available oxygen. In the aerobic aromatic catabolism, oxygen serves both as the terminal electron acceptor and the co-substrate for hydroxylation and oxygenolytic ring cleavage of the aromatic ring. Conversely, anaerobic aromatic catabolism attacks the aromatic ring using a distinct strategy, mainly based on redox reduction [9]. Aerobic aromatic catabolism has undergone extensive

studies, yet anaerobic aromatic degradation research has recently discovered a microbial capacity that is still awaiting an in-depth understanding. Nevertheless, oxygen conditions prevail in many natural and contaminated sites (e.g., submerged soils, aquatic sediments, and aquifers) that are replaced by anaerobic biodegradation using terminal electronic acceptors, such as nitrate, sulfate, or iron ions [64]. Bacteria can induce PAH degradation via the cytochrome P450-mediated pathway as well, with the production of trans-dihydrodiols [65]. or under anaerobic (i.e., nitrate-reducing) conditions [66].

10. Enzymatic degradation mechanisms

Bacteria can cause remediation or degradation to environmental risks through achieving energy sources from almost any compound and have evolved as the ultimate nature over three billion years ago [28]. For PAH decomposition, different bacteria have been discovered, most studied in the naphthalene and phenanthrene decomposition area. Many distinctive metabolic pathways for bacterial PAH decomposition have been well substantiated in several good review articles [67, 68]. PAH can be degraded by some specimens isolated from soil or sediments and a large number of general bacterial species [69]. PAHs in the soil are decomposed by bacterial strains, depending on classified groups such as *Sphingomonas*, *Burkholderia*, *Pseudomonas*, *Mycobacterium*, *sphingomonads*, *Sphingobium*, *Burkholderia fungorum*, *Novosphingobium*, and *Sphingopyxis* [70]. The biochemical pathway of bacterial degradation of LMW PAHs, such as phenanthrene, naphthalene, and anthracene, has been reported [20]. Enzymes involved in PAH decomposition include dehydrogenase, ligninolytic enzymes, and oxygenase. Fungal ligninolytic enzymes include laccase, manganese peroxidase, and lignin peroxidase [54]. Basidiomycete-producing laccases are enzymes that contribute to xenobiotic decomposition. In this study, they were combined to provide an industrial expression operation related to *Lentinula edodes* laccases, determining their enzymatic attribution and estimating their bioremediation competence [71]. Gene models were used to clone the two alleles of the 1573 alkaline lactase alkali from *L. edodes* L54 based on the genome sequence [72]. An alternative laccase-specific signature sequence was introduced by the new upstream consensus (GCTCCGA/CCGGAG). The two alleles were overexpressed in *Pichia pastoris*, purified, and confirmed by zymography. The kinetic analysis offered a catalytic output order, i.e., 2,2'-azino-bis (3-ethyl benzothiazoline- 6-sulfonic), 2,6-Dimethoxyphenol, Guayaquil, L-3,4 dihydroxyphenylalanine, Catechol, and a constant operating temperature range as low as 40°C. Using convenient mediators of 1-hydroxy benzotriazole and 2,2,6,6-tetramethylpiperidine-1-oxyl, recombinant enzymes can mediate the catalysis of a 70-100% decolorization of the dyes selected and anthracene degradation. These results provide a sound basis on which *L. edodes* laccases are used in bioremediations and advances in protein engineering. Table 3 shows the PAH-degrading microorganisms [73].

Table 3. The PAH-degrading microorganisms.

An enzyme (s)	PAH(s)	Degrading microorganisms	Reference
Soluble methane monooxygenases	C ₁ –C ₈ alkanes alkenes and cycloalkanes	<i>Methylococcus sp.</i> , <i>Methylosinus sp.</i> , <i>Methylocystis sp.</i> , <i>Methylomonas sp.</i> , <i>Methylocella sp.</i>	[74]
Particulate methane monooxygenases	C ₁ –C ₅ (halogenated) alkanes and cycloalkanes	<i>Methylobacter sp.</i> , <i>Methylococcus sp.</i> , <i>Methylocystis sp.</i>	[75]

An enzyme (s)	PAH(s)	Degrading microorganisms	Reference
AlkB related alkane hydroxylases	C ₅ –C ₁₆ alkanes, fatty acids, alkylbenzenes, cycloalkanes	<i>Pseudomonas sp.</i> , <i>Burkholderia sp.</i> , <i>Rhodococcus sp.</i> , <i>Mycobacterium sp.</i>	[76]
Eukaryotic P450	C ₁₀ –C ₁₆ alkanes, fatty acids	<i>Candida maltose</i> , <i>Candida tropicalis</i> , <i>Yarrowialipolytica</i> .	[77, 78]
Bacterial P450 oxygenase system	C ₅ –C ₁₆ alkanes, cycloalkanes	<i>Acinetobacter sp.</i> , <i>Caulobacter</i> , <i>Mycobacterium</i>	[79]
Dioxygenases	C ₁₀ –C ₃₀ alkanes	<i>Acinetobacter sp.</i>	[80]

11. Conclusion

In this review, the availability of various analytical equipment to find and determine the amount of biosurfactant produced. A small number of organisms in the endemic microbial flora participate in biosurfactant production in nature to adapt to a variety of harmful conditions. The necessity of biosurfactant purification depends on its applications. For instance, if surfactants are used for environmental remediation, the ultimate yield should be microbial load-free, but at the expense of product quality. For cosmetic and pharmaceutical applications, however, biosurfactants must satisfy different regulatory standards. To heighten biosurfactants for industrial yield remains a challenge. Identifying efficient and significant surfactants is of paramount importance for industrial scale-up because the final yield compound is influenced by nutrients, microorganisms, micronutrients, and environmental factors. To exploit microorganisms in industry, it needs to further understand microbial genetics and physiology.

Funding

This research received no external funding.

Acknowledgments

We thank all colleagues.

Conflicts of Interest

The authors declare no conflict of interest.

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