CHAPTER 6

Utilization of agroindustrial wastes with a high content of protein, carbohydrates, and fatty acid used for mass production of biosurfactant

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1. Introduction

Biosurfactants are normally occurring atoms delivered by microbes. These are amphipathic atoms comprising of both water-loving and water-hating groups that diminish the superficial pressure and structure blends. The biosurfactant produced by different strains are improved by the utilization of insoluble resources [1, 2]. These are delivered by microbes, for example, molds, parasites, and microscopic organisms, during development [3]. The principle worry for biosurfactant creation is the moderately excessive manufacturing rate when contrasted with manufactured surfactants, because of costly crude materials. One technique for decreasing this cost, which for the most part represents 10%–30% of the general expense, is the utilization of elective minimal effort substrates, for example, agromechanical squanders [4]. Besides, if the removal of agromodern wastes is tricky, their valorization as substrates for biosurfactant creation speaks to an extra bit of leeway and abatement in ecological contamination. The benefits of biosurfactants over manufactured surfactants are their biodegradability, low harmfulness, extraordinary selectivity, movement under outrageous temperature, potential of hydrogen (pH) and saltiness, and low basic micellar fixation (CMC).

The biosurfactants upgrade the biodegradation of polycyclic fragrant hydrocarbon mixes in sea oil slicks [5]. In horticultural uses, these assist to expand plant development by evacuating phytopathogens ([6]); in therapeutic research, these are utilized to facilitate the presentation of outside qualities into aimed cells in quality treatment [7]. Ranasalva et al. [8] revealed that these are used in frozen dairy products, especially in ice cream as emulgent and solubility enhancer. The writing portrays different types of waste and by-products from agromodern procedures that are utilized for biosurfactants creation, for example, oil handling waste, starch squander, sugar industry waste, products of soil squander, refinery waste, and creature fat.

Green Sustainable Process for Chemical and Environmental Engineering and Science
https://doi.org/10.1016/B978-0-12-823380-1.00007-1
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Differing microorganisms are known to use various surface-dynamic operators principally so as to adjust and develop an assortment of substrates between normal capacities [9]. These are distributed beneath different development and natural conditions and are principally associated with expanding the solvency and accessibility of different water-immiscible substrates [10]. Organisms from *Rhodococcus*, *Pseudomonas* sp., *Candida*, and *Bacillus* genera are the most broadly ensnared in the creation of various kinds of biosurfactants [11–14]. Biosurfactants display a few points of interest over their substance partners as far as lower toxicity, and more prominent biodegradability and particularity.

2. Biosurfactant types and microorganisms used for its production

2.1 Types of biosurfactant

Biosurfactants are biomolecules comprised of a hydrophobic and a hydrophilic part. The hydrophobic's job depends on long-chain unsaturated fats and hydroxy unsaturated fats. The hydrophilic part is founded on starch, amino eroding, peptides, phosphate, carboxyl corrosive, or alcohol [15].

Microbial surfactants have as of late become a significant result of biotechnology because of their different applications in corrective, restorative, improved oil recuperation, and numerous other fields. The explanation for their prominence as high-worth biotechnological substances is basically in their particular activity of enormous surface pressure-lowering capacity, high frothing, high selectivity, holding explicit movement at higher temperatures, pH, and saltiness, lower harmfulness, higher biodegradability, better natural similarity, relative simplicity of readiness, and broad pertinence [16, 17].

Biosurfactants are biosynthesized by using microbes or yeast with various substrate sources including sugars, oils, and other agrarians such as oil squanders [18]. The basic micelle focus (CMC) of the vast majority of the biosurfactants lies in the scope of 1–200 mg/L and subatomic mass from 500 to 1500 Da [19]. Most microbial surfactants are atoms, containing various structures that incorporate lipopeptides, glycolipids, polysaccharide protein edifices, unsaturated fats, and phospholipids [20,21]. Biosurfactants of various sorts and their qualities are examined in the following sections.

2.2 Fatty acid, phospholipid, and neutral lipids

Most of the microscopic organisms and yeast produce enormous amounts of unsaturated fats and phospholipid surfactants during development on *n*-alkanes. The water-loving (hydrophilic) and fat-soluble (lipophilic) parity (HLB) is straightforwardly identified in the hydrocarbon chain length. These biosurfactants are important for valuable applications in the medical field [16]. Phosphatidylethanolamine delivered by *Rhodococcus erythropolis* developed on *n*-alkane lowers the interfacial strain among water and hexadecane to under 1 mN/m and has a basic micelle fixation (CMC) of 30 mg/L.

Gautam and Tyagi [22] documented that the absence of phospholipid protein is the reason a child stops breathing in premature-birth kids. They have moreover recommended the separation and duplicating the characteristics responsible for surfactant are used in their fermentation design.

2.3 Mannosylerythritol

Mannosylerythritol lipids (MELs) are glycosphingolipids synthesized by many fungal and yeast strains. These MELs have variety of applications in cosmetic industries. MELs have no charged group and are produced by plant-associated growths of the genera Pseudozyma and Ustilago [23,24]. MELs are both water-loving and -hating particles with 4-*O*-β-dimannopyranose erythritol and an unsaturated fat as well as an acetyl bunch as the water-repelling part.

When compared with rhamnolipids and sophorolipids, MELs have several physicochemical properties, for example, blending properties, great biodegradability, and less critical micelle concentration (CMC), physiologic activities, for example, suppressing the development of microbes [25], initiating cell transformation [26,27], separate melanoma cells and human myeloid leukemia cell lines, refining the proficiency of quality transfection [28], and a solid direction capacity with glycol-conjugates of proteins [29,30]. Hence, MELs could be utilized in many enterprises, for example, Safdel et al. [31] suggest pharmaceutical industries, ecological safety, cosmetic, and food industries.

2.4 Lipopeptides and lipoproteins

Various bacterial species produce lipopeptides or peptidolipids, the vast majority of which have significant organic capacities. For example, many have surfactant, antibacterial, or antifungal properties and subsequently have gained interest from industry. They can be comprised of short, straight, or cyclic arrangements of amino acids, connected to an unsaturated fat by means of ester or amide securities or both.

An enormous amount of cyclic lipopeptides, including 10 peptide antimicrobials and lipopeptide antitoxins, have been created. These comprise of a lipid joined to a polypeptide chain. *Bacillus licheniformis* yields a few BS, which are cooperative and show temperature, pH, and salt solidness. These are additionally comparable in auxiliary and physico-concoction properties to surfactin. The surfactants created by *B. licheniformis* are able to lower the surface pressure of water to 27 mN/m and the interfacial strain among water and *n*-hexadecane [16].

Mass spectrometry and high-vitality couple mass spectrometry uncovered that the acyl chain length in a surfactin structure can fluctuate from 12 to 15 carbons. The dissolvability and surface-dynamic properties are subject to the direction of the deposits. Notwithstanding, yields are commonly low for surfactin (0.02 g/g glucose). Iron and manganese salt expansion could upgrade yield of surfactin up to 0.7 g/L.

Cyclic surfactins embrace a compliance with the end goal that the unsaturated fat chain remains collapsed on the peptide. The collapsing is represented by the expanded intramolecular hydrophobic associations with lipopeptide chain length. These perceptions in this manner give knowledge toward the collaboration of surfactin with the lipid films [32]. Surfactin displays "cleanser-like" activity on cell layers because of its vague system of film permeabilization at fixations under the CMC esteems [33]. A large group of fascinating highlights of surfactin has opened different roads for its applications, for example, antimicrobial, antiviral operator, against cement and calming uses [34]. Surfactin has likewise been seen as a functioning operator in biodegradation of pesticides.

2.5 Polymeric biosurfactant

High atomic weight biopolymers for the most part display helpful properties, for example, high consistency, rigidity, and protection from shear. It is, in this manner, not surprising that polymeric biosurfactants have discovered an assortment of modern uses. The best-comprehended polymeric biosurfactants are liposan, alasan, emulsan lipomanan, and other polysaccharide–protein edifices.

Acinetobacter calcoaceticus RAG-1 incorporates a solid polyanionic amphipathic heteropolysaccharide bioemulsifier. Emulsan is a ground-breaking emulsifying expert for hydrocarbons, indeed, even at a level of >0.001%–0.01% [16].

(a) Emulsan: Emulsan is a polyanionic amphipathic heteropolysaccharide. Emulsan doesn't apparently diminish interfacial pressure, making it an effective emulsifier for hydrocarbons emulsions. The most impressive emulsion stabilizers oppose reversal at a water and oil proportion of 1:4.

Biodispersan: A. calcoaceticus A2 has been accounted to deliver an extracellular, non-dialyzable scattering specialist called biodispersan. The dynamic part of biodispersan is an anionic heteropolysaccharide with a normal atomic load of 51,400 and 4 decreasing sugars, in particular, glucosamine, 6-methyl aminohexose, galactosamine uronic corrosive, and an unidentified amino sugar.

(b) Liposan: Extracellular water-dissolvable emulsifier, assigned as liposan, is incorporated by *Candida lipolytica*. It is made out of 83% sugar and 17% protein [35]. The starch partition is a heteropolysaccharide comprising of galactose, glucose, galactosamine, and galacturonic corrosive.

2.6 Rhamnolipids

Rhamnolipids are commonly used glycolipids biosurfactants that have a variety of use in the bioremediation cleaning or washing, skin, and hair care industry [36]. It additionally has antimicrobial, antiadhesive, and antibiofilm properties. Rhamnolipids have assorted and characteristic molecular construction with one or two rhamnose, 3-hydroxy fatty

contains 8–14C [37]. Rhamnolipids, as created by *Pseudomonas aeruginosa*, is a gathering of biosurfactant that has been concentrated broadly in olive mill leftover as nutrients [38].

A few physical and chemical properties are vital parts components to calculate the surface and interfacial tension, molecule size, critical micelle concentration (CMC), and solubilizing limit. Rhamnolipids have truncated surface tension (28–30 mN/m), extraordinary mixing action (up to 60%–70%), and low CMC (10–200 mg/L).

2.7 Sophorolipids

Sophorolipids (SLs) are glycolipid biosurfactants richly delivered from various feedstocks by yeasts, and have been broadly produced for different applications. This biosurfactant is produced using yeast with glucose and olive fat as effective nutrients [39]. It can also be produced by *Candida bombicola* utilizing lignocellulosic feedstock as a carbon source. [40]. Sophorolipids are produced by a few yeasts of animal types, for example, Wickerhamiella domercqiae, *Candida* sp., *Pichia anomala*, *Cryptococcus* sp., Rhodotorula bogoriensis, etc. Basically, they comprise of a dimeric sugar d with a long chain of hydroxyl fat, connected by a β-glycosidic security [39,41,42].

Sophorolipids have been exhibited to be compelling anticancer operators against malignant cell lines [7]. These lipids are created on a business scale when an organism is refined on substrates containing glucose and a wellspring of alkyl moieties, for example, alkanes or seed oils, which affect the unsaturated fat constituent. Yield of sophorolipids can be as high as 300 g/L from living beings during the fixed stage.

3. Metabolic pathway

The pathway of microbial biosynthesis of a surfactant is shown in Fig. 1. A hydrophilic substrate, glucose 6-phosphate, the principle originators of starches are found in BS. Here, the glucose is oxidized to pyruvate, then changed over into acetyl CoA and yields malonyl CoA, after joining with oxaloacetate, trailed through transformation into an unsaturated fat, the originators for the combination of lipids. The metabolic pathway of gluconeogenesis is enacted for the making of sugars. This comprises the oxidized unsaturated fats to acetyl-CoA. The development of acetyl-CoA, the responses engaged with the amalgamation of polysaccharide foreshadowing, for example, glucose 6-phosphate, basically is the reverse of that associated with glycolysis. In this manner, different catalysts selective to the procedure of gluconeogenesis are essential to dodge such responses. The figure outlines the primary responses to the development of glucose 6-phosphate, the principle precursor of poly- or di-saccharides shaped for the creation of glycolipids structural arrangements. The surfactant biosynthesis happens through four distinct highways: (a) starch and lipid combination; (b) union of the sugar while the union of the lipid relies upon the carbon chain length in the medium; (c) union of the lipid while the amalgamation of the carbon relies upon the substrate utilized; and (d) a blend of the carbon and lipid parts. Hence, the

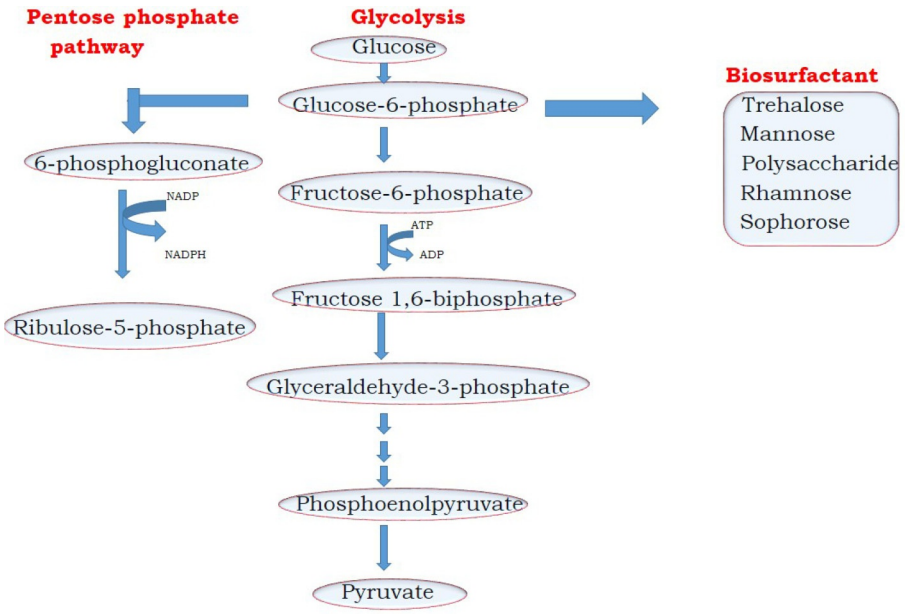


Fig. 1 Biosynthesis of microbial pathway of surfactant.

n-alkane chain length utilized as the carbon source changes the surfactant biosynthesis. Kitamoto et al. [43] contemplated the formation of manosilerythritol lipid (MEL) by *Candida antarctica* within the sight of various *n*-alkanes, and this species doesn't develop a biosurfactant in media comprising C10 to C18.

4. Production of biosurfactant using wastes from agroindustries

Generally, a biotechnological process in large scale involves high-cost contributions, so it is important to certify the finest product performance at the most reduced expenditure through inexpensive resources. Then again, microorganisms synthesize extremely low measures of dynamic tense, and biotechnological items cost somewhere in the range of 60% and 80% of the complete creation consumption; subsequently, it is important to decrease manufacturing costs through the use of commercial and renewable resources [9].

The agroindustrial wastes contain high sugar and fat content used as a substrate for producing many products, even biosurfactant. In large-scale production of biosurfactant, these substrates are cost-effective, and their measured economic and environmental disposal can be minimized. The reutilizing and retrieval of wastes from the lubricant industries, distiller, dairy industries, food-processing, and carbohydrate wastes are proposed for BS production [9,22].

4.1 Starchy vegetables

Saravanan and Vijayakumar [44] used a bacterial strain *P. aeruginosa* PB34 for biosurfactant production by using agricultural waste such as rice fiber, potato waste, peanut cake, barley fiber, and cassava flour waste on replacing carbon source glucose. Most of the agricultural waste is in a large amount, so these can be used as nutrients for the growth of organisms along with organic salts, inorganic salts, and nitrogen sources.

Fox and Bala [45] featured the expected natural hazard and monetary obligation of starch-rich wastes from potato-preparing ventures. Potatoes are made out of water, protein, sugars, fat, and nutrients (80%, 2%, 17%, 0.1%, and 0.9%); inorganic minerals; and minor components. The carbon, nitrogen, sulfur, and minerals are minor components and nutrients. Cassava wastewater, another sugar-rich buildup, which is created in huge sums during the readiness of cassava flour, is additionally an appealing substrate and is utilized for surfactin creation by *Bacillus subtilis* [20]. A few other dull waste substrates, for example, rice water, corn steep alcohol, and oats, beats wastewater are can possibly bolster microbial development and biosurfactant creation [35].

Rural wastes, for example, grain wheat, cutting corn cobs, plant shoots, and eucalyptus have been used for the synchronous formation of biosurfactant and lactic corrosive [46]. Ramnani et al. [47] used starch of potato, corn, and rice alongside solvent starch and lactose as carbon for the creation of biosurfactant using *B. licheniformis* RG1.

Zhu et al. [48] used rice straw and soybean flour for the lipopeptides production with the help of *Bacillus amyloliquefaciens* XZ-173. By using solid substrate fermentation (SSF) process, the lipopeptide were produced in large amounts because of the optimized medium that contains yeast extract (1.9%), starch (1.8%), soybean flour (5.6 g), and rice hay (3.7 g). Strong antimicrobial activity against *Ralstonia solanacearum* and *Rhizoctonia solani* was discovered. Freitas Silva et al. [49] scrutinized the medium that contains corn steep liquor 8% and rice fiber 3% that increased the production of biosurfactant using *Rhizopus arrhizus*. Oje et al. [50] assessed the influence of acid and alkaline pretreatment on rice husks using *Mucor indicus* for glycolipids production. Their results showed that the medium with rice husk, $NaNO_3$, and mineral salts with alkaline pretreated rice shells increased the yield of glycolipid. For the lipopeptide production, the cassava wastewater (2.2 g/L) can enhance the growth of *subtilis* ATCC 21332 and can favor the production process. *Pseudozyma tsukubaensis* [51] utilized cassava waste for the production process. The biosurfactant can be produced using *R. arrhizus* with rice bran husk as substrate [49].

4.2 Molasses

In another investigation with agroindustrial wastes (corn steep alcohol and molasses), rhamnolipids showed a superior presentation in eliminating oil from polluted sand when compared with enordet and petrostep, which are chemical surfactants. Molasses is a result

of the sugar business and is a significant crude material for the creation of liquor, bread cook's yeast, citrus extract, feed yeasts, $(CH_3)_2CO$, natural acids, and amino acids. The molasses is relatively low in value contrasted with different sources of sugar and is wealthy in different supplements other than sucrose. Normal qualities for the constituents of stick molasses are all-out sugars, nonsugar natural issue protein, potassium, calcium, magnesium, phosphorus, biotin, pantothenic corrosive, inositol, and thiamine.

The biosurfactant was produced by *Pseudomonas fluorescens* and it utilized molasses as a carbon source. Biosurfactant formation was confirmed by estimating optical thickness (OD), surface pressure, and emulsifying file (E24) more than 12 days of aging. The ideal condition for the biosurfactant production was acquired when a medium contained nutrient broth, NaCl, NH_4NO_3, and pretreated molasses. The biosurfactant was distinguished as a rhamnolipid-type biosurfactant that had critical micelle concentration (CMC) estimation of 801 mg/L and had the option to decrease the surface tension from 80 mN/m to 51 mN/m [52]. Molasses and corn steep alcohol are utilized as the sole wellspring of carbon and nitrogen for rhamnolipid biosurfactant production from *P. aeruginosa* GS3. Rhamnose grouping of 0.25 g/L and decrease of interfacial strain among surfactant and raw petroleum of 0.47 mN/m was observed [53]. Biosurfactant creation was investigated by *B. licheniformis* K51, *B. subtilis* R1, *B. subtilis* 20B, and *Bacillus* strain HS3 utilizing molasses sustenance.

4.3 Animal fat waste

Animal fat are acquired in enormous amounts from meat-handling ventures and have been utilized as a preparing mode for food. Animal fat was used for sophorolipid biosurfactant production by yeast, *C. bombicola*. When utilizing just fat as carbon source, the development was reduced; be that as it may, a blend of glucose (10%) and fat (10%) brought about the most elevated level of development. The sophorolipid was produced using pH control separately.

Santos et al. [54] understood that the medium with bovine fat (5%) along with corn steep liquor (2.5%) can increase the growth of *C. lipolytica* UCP0988 and also the productivity is highest with agitation. Later these biosurfactants are also investigated in clear-out oil spills at coastlines and sea [54] and also for heavy metals and pollutants removal. The bacteria *B. subtilis* PF1 consecutively produced biosurfactant, keratino-lytic protease, and amylase. The high yield was obtained based on the production medium with chicken feather (12.5 g/L), potato peel (12.5 g/L), and rape seed cake (6 g/L). These biosurfactants are used in laundry detergents, which are stable in varied temperature and pH.

Minucelli et al. [55] assessed the sophorolipids using *C. bombicola* ATCC 22214 on utilizing chicken fat, sucrose or glucose, sunflower oil, sugarcane molasses, and juice. The sophorolipids produced under ideal states of glucose (77.5 g/L), chicken fat

(75 g/L), and yeast (2.5 g/L) separate. As of late, Martins and Martins [56] worked with *Corynebacterium* sp. CCT 1968 for the synthesis of biosurfactants utilizing modern waste such as fish squander (skin, heads, scales, muscles, bones, and viscera), sugarcane bagasse, rough glycerol, and oil ooze from capacity tanks.

4.4 Whey

The overflow from dairy wastewater underpins great microbial development and has been utilized as a modest crude material for BS. Whey squander was used for the development of *P. aeruginosa* BS2 inside 48 h. The isolated biosurfactant had powerful surface dynamic properties, as it successfully diminished the surface strain of water. Societies developed on refinery and whey squander gave biosurfactant (0.91 g/L) separately. Curd whey was used as a vehicle for the financially savvy creation of biosurfactant via *Kocuria turfanesis* strain-J, *P. aeruginosa* strain-PP2, and the BS delivered was successful considerably under outrageous pH and temperature conditions [57].

The capability of *B. subtilis* ANR 88 was examined for the insignificant medium with potato peels, orange peels, bagasse, and banana peels, just as molasses and whey. After enhancing the medium with molasses (4%) and ammonium ferric citrate (0.25%), the lipopeptides production improved from 0.24 to 0.746 g/L. The lipopeptide was powerful in the amalgamation of gold and silver nanoparticles without the involvement of chemicals.

Utilizing cassava flour wastewater (CWW), carbon, and whey, de Andrade et al. [58] enhanced the surfactin production using *B. subtilis* LB5a. It showed that the best substrate was whey, activated carbon, and CWW (28 g/L, 25 g/L, and 74 g/L). These results indicate a significant strategy for the production of biosurfactant using cost-effective substrates. de Andrade et al. [51] assessed the MEL-B production using *P. tsukubaensis*, which utilizes 3 L cassava flour wastewater. The purified form of MEL-B was obtained by ultrafiltration process.

4.5 Vegetable oil waste

Cooking oil typically stays fluid at 37°C temperature, which is palatable. Squandered cooking oil from family unit usage and food manufacturing is fowled after being heated over 150–190°C temperature [59]. WCO is a critical water toxin, and its removal comprises a urban wastewater issue because of its low dissolvability in water, influencing natural wastewater management, and thus influencing microorganisms, plants, and sea-going biological systems [60]. So by using WCO as a substrate, biosurfactants are produced in a large amount.

Plant-based oils, rapeseed [61], corn, and babassu oil have been confirmed to be helpful as modest crude materials for BS production. Indistinguishably, vegetable oils are

utilized for sophorolipid, rhamnolipid, and mannosylerythritol lipid biosurfactants production utilizing different organisms [62]. Aside from different vegetable oils, oil wastes from vegetable-petroleum processing plants or cleanser enterprises and the food business are additionally detailed as great substrates for biosurfactant creation [38,63].

Nut oil cake, a by-product delivered in enormous amounts during the making of nut oil, is comprised of high sugar, protein, and lipid content, and is generally modest. Thavasi et al. [64] utilized nut oil cake as a nutrient for the creation of biosurfactant utilizing microorganisms *Lactobacillus delbrueckii*. The biodegradation of unrefined petroleum was elevated to a huge degree by biosurfactant alone delivered using nut oil cake as a substrate without the utilization of compost. Zhu et al. [48] used waste singing oil for the development of three strains of *P. aeruginosa* to deliver rhamnolipids as biosurfactants. The biosurfactant by *B. subtilis* LSFM-05 was completed with crude glycerol as the sole wellspring of carbon, acquired from a biodiesel plant in Brazil [65].

The basic biosurfactant by using *P. aeruginosa* ATCC27853 (strain A) [66] has given higher yield when compared with other species like Bacillus and Pseudomonas. Nazren et al. [63] detailed that *P. aeruginosa* gave good yield of biosurfactant (0.43 g/L) on utilizing carbon source (palm unsaturated fat) PAO1, although Moya et al. [38] watched *B. subtilis* and *P. aeruginosa* on utilizing olive oil. Benincasa and Accorsin [67] worked with *P. aeruginosa*, which gave 4.9 g/L BS yield using carbon source. e Silva et al. [4] revealed a biosurfactant (5.2 g/L) formation utilizing waste cooking oil.

5. Purification and recovery of products

5.1 Purification of biosurfactant

The fermentation of biosurfactant has been improved to obtain maximum yield. The downstream process is quite expensive with almost 60% of production cost, even though the purity is not satisfied when it comes to pharmaceutical and cosmetic applications. Few past investigations show adsorption, foam fractionation, membrane filtration, and a combination of any two for extracting BS. In industrial routines, these methodologies are ineffective and have low feasibility [68,69]. Apart from these techniques, foam fractionation, ion exchange chromatography, ultrafiltration, and adsorption resins are some of the alternative recovery process of biosurfactants [47,70]. Based on the biosurfactant properties, these techniques are useful for the large-scale purification of biosurfactant.

5.1.1 Adsorption

After the removal of biomass, adsorption process was carried out using XAD-2 resin for the extraction of rhamnolipids, then elution was achieved by the addition of ethyl acetate/ isopropanol. For the adsorption process, XAD-2 resin bed was 10.4 L as volume, and for equilibrating the adsorption bed, 0.1 M potassium phosphate buffer was used. The

purity of rhamnolipids was about 60% after the adsorption process [71]. In this experimental study, the novel biosurfactant was introduced into the absorbents Ti_2CT_X MXene for the elimination of metals like Pb(II) ions. The retention time of metal ions to the absorbent plays an important factor, and about 90% of ions removal rate was attained. Lastly, the adsorption attains equilibrium in 24 h, then the solution is filtered using filter paper, which contains concentrated Pb(II) [72]. The cation, anion, and neutral biosurfactants are separated by using nanosheets made up of graphene oxide (GO). There is electrostatic and hydrophobic interaction between GO and biosurfactants, even SDS, Triton X-100, and trimethyl ammonium bromide (DTAB). The adsorption patterns for different biosurfactant and GO were proposed [73].

5.1.2 Foam fractionation

In foam fractionation, the feed was introduced at the top of the column where the growing foam presents. The wet growing foam is seen below the feed point, and above it the foam drains and is dried, which results in the retentate collection from lower end and foamate from column top. For the separation of rhamnolipid, fractionation process was accomplished with airflow (0.1, 1.2 L/min) rates. This process was achieved based on the size of the foam, foam stability, and foam structure. The rhamnolipid concentration was scrutinized based on the feed, retentate, and foamate samples [74]. After the production of lipopeptides from *Bacillus* sp. GY19 using medium with oil and unwanted glycerol, biosurfactant of about 10.9 and 9.8 g/L were extracted from the culture broth using foam fractionation process. Instead of palm oil, the oil displacement efficiency was absolute using diesel oil, Arabian light oil, and heavy oil [75].

5.1.3 Ultrafiltration

Ultrafiltration is used for the recovery of suspended particles based on the molecular size, which contains a membrane for the separation process. About 95% of biosurfactant are recovered from culture broth using ultrafiltration with 100 kDa membrane. It is a cost-effective single-step method. The biosurfactant and the enzyme are recovered by adding sodium sulfate, which tends to aggregate and retain in the membrane. This dried slurry is used as an additive in detergent industries. The biosurfactant produced by *Bacillus methylotrophicus* and *Bacillus pumilus* is purified using membrane separation processes called ultrafiltration. This process was more effective for the purification of surfactin that was retained in the membrane [76].The cost-effective production of surfactin with cassava wastewater are purified using ultrafiltration membrane (PES-100 kDa). Before this process, unwanted proteins were removed by acid precipitation and extraction process, which improved the purity of the product [58].

5.1.4 Ion change chromatography

B. subtilis was isolated from five different areas of Iraq for the production of biosurfactant. These crude biosurfactants are purified by dichloromethane and then by two steps of purification with ion exchange chromatography and size exclusive chromatography (Sepharose-6B) [77]. From the local market of Iraq, *Saccharomyces cerevisiae* was isolated, which produced mannoproteins (biosurfactant) using YPEG medium. The mannoproteins were purified by three different steps: first, treated with mercaptoethanol and sodium dodecyl sulfate, then second, by using chromatography. The purified mannoprotein shows 80% emulsion activity, and the surface activity was 37 mm [78]. For rhamnolipid purification process, ion change chromatography CL 6B (bed volume 6.2 L) column, 10 mM tris-HCL buffer (pH 8), and 10% ethanol was used. About 5 g/gel was recovered from these four steps of purification process, and the product obtained was 90% pure. The downstream processing of this procedure first removes the biomass, then adsorption process, ion exchange chromatography, and again the solution undergoes adsorption process to obtain pure form of rhamnolipids [71].

5.2 Identification of recovered biosurfactant

5.2.1 Thin layer chromatography

Thin layer chromatography (TLC) is the common method for the refining process, as it is a quick, simple, and cost-effective method [79,80]. For the identification of biosurfactant, TLC was used, which has a mobile phase and stationary phase. The production of *Bacillus* surfactin lipopeptides were analyzed with altered solvents and with varying concentrations of sample; in the TLC plates, multiple spots of surfactin were identified (98% accuracy) [81]. The biosurfactant separated from two different medium sources, such as glucose and molasses, were analyzed using HPTLC, but the retention factor (Rf) was similar. The band intensity of biosurfactant from glucose-rich medium was high based on the concentration. A few specialists detailed utilization of TLC or HPTLC as a device for beginning subjective recognition or quantitative examination of various biosurfactants [82].

5.2.2 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) is used to determine the atoms/molecules on applying a magnetic field; the nucleus absorbs radiofrequency and spins to flip to higher energy direction. These nuclei adsorb energy, remits the radiation, and returns to ground state. NMR discloses the functional groups and also the position of bond between the carbohydrate or lipid molecules. Solvents used in NMR studies are acetone, benzene, chloroform, dimethyl sulfoxide, and methanol [33]. From Lake Chilika, *Bacillus tequilensis* CH isolated for the production of biosurfactant were partially characterized by using thin-layer

chromatography (TLC), nuclear magnetic resonance (NMR) techniques, and Fourier transform infrared spectroscopy (FTIR) [83]. Agroindustrial waste such as soybean leftover cooking oil (2%, carbon) and CSL (2%, nitrogen source) are used for the BS from *Pseudomonas cepacia* CCT6659. *Pseudomonas* sp.-producing biosurfactant are mostly glycolipids, which were confirmed by NMR analysis (^{1}H and ^{13}C NMR) [84].

5.2.3 High-performance liquid chromatography

In bacterial cultures, the lipopeptides pseudofactin and surfactin were quantified using UPLC and high performance liquid chromatography (HPLC) [38]. In HPLC system, the retention time and peak areas are important to quantify the samples, which take 15–20 min of running time per sample including column wash and equilibrium process. This method of quantification and characterization biosurfactant are very accurate, sensitive, conventional, and repeatable [38,85]. The lipopeptide synthesized by *Bacillus thuringiensis* pak2310 after 6 days of fermentation process was separated and purified. The lipid, water-soluble antibiotics were extracted using solvents, refined using HPLC analysis, and gives three peaks from 5 to 9 min of total run. The *B. thuringiensis* strains produce purified cyclic lipopeptide surfactants (100 mg/mL) [86].

5.2.4 Electrospray ion mass spectroscopy

For the biosurfactant characterization, electrospray ion mass spectroscopy (ESIMS) and secondary ion mass spectrometry (SIMS) are used, which analyze the subatomic weight per charge (m/z) assessment. The biosurfactant lichenysin, a lipopeptide, was scrutinized by the positive- and negative-particle SIMS and ESIMS [87,88]. The isomers of surfactin were detected online by a rapid method, which was advanced based on analyses of HPLC-ESIMS. The NMR and ESI-MS were used to identify the stereostructures of biosurfactants. *P. aeruginosa* NCIM 5514 produced rhamnolipids (RL) that were analyzed by mass spectrometry, which shows the biosurfactant molecular masses at 476 m/z and 703 m/z respectively. The mobile phases used are ammonium acetate (0.05%), formic acid (0.1%): methanol (100%) (80:20, v/v) at flow rate 0.3 mL/min. The temperature for H-ESI was fixed to 200°C at 2 kV [14,37,89]. *Bacillus* spp. produced lipopeptides (surfactin, iturin, and fengycin) shows antifungal activity and hemolytic activity that were analyzed by ESI-MS. The microorganisms such as *B. subtilis* NH-100 and *Bacillus atrophaeus* 176s were filtered to obtain the production of biosurfactants. The analysis shows three intense peaks are of surfactin and fengycins, the low intense peaks shows iturin presence at m/z 1008, 1030.8, 1044.8, and 1058.6 and at m/z 994.8, 1008.8, 1022.8, and 1050.6. These peaks confirmed the presence of isomers with C_{12}, C_{13}, C_{14}, C_{15}, and C_{16} acyl chains [90].

6. Application of biosurfactant

Agrowaste/ substrate	Microbes	Biosurfactant types	Yield (g/L)	Application	References
Rice husk	*M. indicus*	Glycolipids	7.8	Spilled oil remediation	[50]
Lignocellulose hydrolysates	*B. tequilensis* ZSB10	Biosurfactants	1.52	Ability to emulsify kerosene	[91]
	S. bombicola NBRC	Sophorolipids	49.2	Enhance oil Recovery	[39]
	C. bombicola ATCC 22214	Sophorolipids	52.1	In cosmetic, hygienic, and pharma codermatological products as bactericidal agent	[92]
	C. mucoides UFMG-CM-Y6148	Sophorolipids	12.5	Biotensoactives or bioemulsifiers, antimicrobial, and anticancer agent	[40]
Animal fat and animal waste	*P. aeruginosa* 101045	Rhamnolipids	3.84	Bioremediation	[93]
	C. lipolytica UCP0988	Glycolipids	8	In food industries as thickeners, foaming agents, humectants, and emulsifying agents	[54]
	C. bombicola ATCC 22214	Sophorolipids	39.8	Bioremediation	[55]
Vegetable oil processing waste and byproducts	*C. sphaerica* UCP 0995	Glycolipids	21	In agriculture and food industries	[94]
	B. subtilis SPB1	Lipopeptides	30.67 (mg/g)	As antibacterial agent, Fungicide effect	[95]
	P. aeruginosa PAO1	Rhamnolipids	0.1914	In food business, oil recovery practices and therapeutic uses	[38]
	T. versicolor	Biosurfactants	373.6 (mg/ 100 g)	As cleansing agent, drenching and frothing agents, solubility enhancement, emulgent and dispersing agent	[96]
	S. bombicola MTCC 1910	Sophorolipids	51.5	Emulsifying and antioxidant activity	[97]

Agrowaste/ substrate	Microbes	Biosurfactant types	Yield (g/L)	Application	References
Frying oil waste	*P. tsukubaensis*	Mannosynthritol lipids-B	1.26	In pharmaceutical industries (as antitumor drug), biodegradation of petroleum, cosmetic industry	[51]

7. Conclusion

Biosurfactants have vast applications in food, cosmetics, and pharmaceutical industries and for environmental applications. These biosurfactants are fermented from complex sugar molecules with the help of microorganisms. The agroindustrial waste is polluting the environment, so these are utilized by microorganisms as substrates for the production of biosurfactants. The most important class of biosurfactant is surfactin, which has a specific role in the recovery of oil. The agroindustrial waste such as starchy vegetables, molasses, animal fat waste, whey, and plant-based oil waste are used as nutrients for microbial surfactant. These substrates are rich in sugars and nutrients, and are a cost-effective method for biosurfactant synthesis. The growing demand and commercial value of biosurfactant could in a way be the driving force for transformation of rural economics into industrial ones.

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