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A Review on Biosurfactants: Fermentation, Current Developments and Perspectives

***BS Saharan[1], RK Sahu[2], D Sharma[1]**

[1]Department of Microbiology
Kurukshetra University, Kurukshetra
Haryana 136 119, INDIA

[2]The Center For Autoimmune and Musculoskeletal Disease
The Feinstein Institute for Medical Research
350 Community Drive
Manhasset, New York 11030

***corresponding author**

Abstract

Surfactants are compounds that reduce the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. Surfactants are characteristically organic compounds containing both hydrophobic groups (their *tails*) and hydrophilic groups (their *heads*). Therefore, a surfactant molecule contains both a water insoluble (and oil soluble component) and a water soluble component. Biosurfactnats encompass the properties of dropping surface tension, stabilizing emulsions, promoting foaming and are usually non-toxic and biodegradable. Interest in microbial surfactants has been progressively escalating in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under intense circumstances and their impending applications in environmental fortification. These molecules have a potential to be used in a variety of industries like cosmetics, pharmaceuticals, humectants, food preservatives and detergents. Presently the production of biosurfactants is highly expensive due to the use of synthetic culture media.

Therefore greater emphasis is being laid on procurement of various cheap agro-industrial substrates including vegetable oils, distillery and dairy wastes, soya molasses, animal fat, waste and starchy waste as raw materials. These wastes can be used as substrates for large-scale production of biosurfactants with advanced technology which is the matter of future research. This review article represents an exhaustive evaluation of the raw materials, with respect to their commercial production, fermentation mechanisms, current developments and future perspectives of a variety of approaches of biosurfactant production.

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* Corresponding author. Tel.: +91-9416545332.
E-mail address: Baljeet.kuk@gmail.com (Baljeet Singh).

Keywords

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1. Introduction

Surfactants are amphiphilic surface active agents possessing both hydrophilic and hydrophobic moieties that reduce surface and interfacial tensions by accumulating at the interface between two immiscible fluids like oil and water, signifying that surfactants moreover assist the solubility of polar compounds in organic solvents. They are of synthetic or biological origin. Due to their interesting properties such as lower toxicity, higher degree of biodegradability, higher foaming capacity and optimal activity at extreme conditions of temperatures, pH levels and salinity, these have been increasingly attracting

the attention of the scientific and industrial community. Biosurfactants are a group of structurally diverse molecules produced by different microorganisms classified mainly by their chemical structure and microbial origin. Structurally, they contain a hydrophilic moiety, comprising an acid, peptide cations, or anions, mono-, di- or polysaccharides and a hydrophobic moiety of unsaturated or saturated hydrocarbon chains or fatty acids. They are mainly classified into two classes: low-molecular weight surface active agents called biosurfactants (lipopeptide, glycolipids) and bioemulsifiers (high molecular weight surface active agents). They efficiently reduce surface and interfacial tensions [1, 2]. Biosurfactants are further divided into six classes: hydroxylated and cross linked fatty acids (mycolic acids), glycolipids, lipopolysaccharides, lipoproteins-lipopeptides, phospholipids and the complete cell surface itself.

Biosurfactants have many environmental applications such as bioremediation and dispersion of oil spills, enhanced oil recovery and transfer of crude oil. Other potential applications of biosurfactants relate to food, cosmetic, health care industries and cleaning toxic chemicals of industrial and agricultural origin.

Objectives of this Review

- To assess the current perspectives of biosurfactant production using inexpensive and easily available agro-industrial substrates.
- To provide an insight into the role of various developed processes like fermentation, optimization, product recovery, substrate utilization and major controls that may be used for their management in production of cost effective microbial surfactants.

- To highlight the limitations and challenges related to the various developed processes of biosurfactant fermentation.

2. Microorganisms producing biosurfactants

Biosurfactants produced by a variety of microorganisms mainly bacteria, fungi and yeasts are diverse in chemical composition (Table-1) and their nature and the amount depend on the type of microorganism producing a particular biosurfactant. Many microorganisms for industrial utilization for waste products have been isolated from contaminated soils, effluents and waste water sources. Thus, these have an ability to grow on substrates considered potentially noxious for other non-producing microorganisms.

Table-1: List of biosurfactant producing organisms.

Sr. No.	Biosurfactant	Microorganism(s)	Current economic importance	Reference(s)
1.	Cellobiose lipids	*Ustilago maydis*	Antifungal Compounds	[3]
2.	Serrawettin	*Serratia marcescens*	Emulsification of hydrocarbons	[4]
3.	Polyol lipids	*Rhodotorula glutinis, R. graminis*	Anti-proliferative activity	[5]
4.	Trehalose lipids	*Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia erythropolis, Corynebacterium* sp., *Mycobacterium* sp	Dissolution of hydrocarbons	[6]
5.	Ornithine lipids	*Pseudomonas* sp., *Thiobacillus thiooxidans, Agrobacterium* sp.	Bio-emulsifiers	[7]
6.	Viscosin	*Pseudomonas fluorescens, Leuconostoc mesenteriods*	Surface active lipopeptides	[8]
7.	Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas chlororaphis,Serratia rubidea*	Bioremediation, Antimicrobial and biocontrol properties	[9]
8.	Carbohydrate-lipid	*P.fluorescens, Debaryomyces polmorphus*	Bio-emulsifiers	[10]
9.	Protein PA	*P.aeruginosa*	Bio-emulsifiers	[11]
10.	Diglycosyl diglycerides	*Lactobacillus fermentum*	Bio-remediation	[12]
11.	Whole cell	*Cyanobacteria*	Bio-flocculent	[13]
12.	Fatty acids /neutral lipids	*Clavibacter michiganensis* subsp. *insidiosus*	Bio-emulsifiers	[14]
13.	Sophorolipids	*Candida bombicola, C. antartica, Torulopsis petrophilum C. botistae, C. apicola, C. riodocensis, C. stellata,*	Antimicrobial, Antiviral, Spermicidal	[15]

		C. bogoriensis		
14.	Liposan	*C. tropicalis*	Bio-emulsan	[16]
15.	Monnosylerythritol lipids	*C.antartica, Kurtzmanomyces* sp., *Pseudozyma siamensis*	Antifungal compounds	[17]
16.	Surfactin/Iturin	*B. subtilis, B. amyloliquefaciens*	Antimicrobial properties	[18]
17.	Subtilisin	*B. subtilis*	Antimicrobial properties	[19]
18.	Aminoacids lipids	*Bacillus* sp.	Antimicrobial properties	[20]
19.	Lichenysin	*Bacillus licheniformis, B. subtilis*	Microbially enhanced oil recovery (MEOR)	[21]
20.	Peptide lipids	*B. licheniformis*	Antimicrobial properties	[22]
21.	Phospholipids	*Acinetobacter* sp.	Bioremediation	[23]
22.	Vesicles & fimbriae	*Acinetobacter calcoaceticus, P. marginilis, P. Maltophila*	Bioremediation	[7]
23.	Emulsan	*A. calcoaceticus*	Microbially enhanced oil recovery (MEOR)	[24]
24.	Alasan	*A. radioresistens*	Biodegradation of polyaromatic compounds	[25]

3. Economic factors of Biosurfactant production

To overcome the expensive cost constraints associated with biosurfactant production, two basic strategies are generally adopted worldwide to make it cost-effective: (i) the use of inexpensive and waste substrates for the formulation of fermentation media which lower the initial raw material costs involved in the process; (ii) development of efficient and successfully optimized bioprocesses, including optimization of the culture conditions and cost-effective recovery processes for maximum biosurfactant production and recovery. As millions of tons of hazardous and non-hazardous wastes are generated each year throughout the world, a great need exists for their proper management and utilization. The residues from tropical agronomic crops such as cassava (peels), soybeen (hull) [26], sugar beet [27], sweet potato (peel and stalks), potato (peel and stalks), sweet sorghum [28], rice and wheat [29] bran and straw); hull soy, corn and rice; bagasse of sugarcane and cassava;

residues from the coffee processing industry such as coffee pulp, coffee husks, spent coffee grounds; residues of the fruit processing industries such as pomace and grape, waste from pineapple and carrot processing, banana waste; waste from oil processing mills such as coconut cake, soybean cake, peanut cake, canola meal and palm oil mill waste; saw dust, corn cobs, carob pods, tea waste, chicory roots etc. have been reported as substrates for biosurfactant production [30]. Additional substrates used for biosurfactant production include water-miscible wastes, molasses, whey milk or distillery wastes. [28].The various substrates previously reported for biosurfactants production are listed (Table-2) with their advantages.

Table 2: Substrate for Microbial surface active agents and their advantages

Source	Substrate part	End product (s)	Reference(s)
Cassava	Flour	Biosurfactant	[31]
Soybean oil	Seeds	Rhamnolipid	[26]
Sugar beet	Peels	Biosurfactant	[27]
Sweet Potato	Peels	Biosurfactant	[28]
Sweet Sorghum	Peels	Biosurfactant	[28]
Rice and wheat bran	Stem Husk	Biosurfactant	[32]
Sugarcane Bagasse	Stem Husk	Biosurfactant	[29]
Cashew Apple juice	Pomace	Biosurfactant	[33]
Dairy Whey	Whey	Bioemulsifiers	[34]

4. Substrates for commercial microbial production

Despite possessing many industrially attractive properties and advantages compared with

synthetic ones, the production of biosurfactants on industrial scale has not been undertaken due to high investment costs. This necessitates their profitable production and recovery on a large scale. Various aspects of biosurfactants, such as their biomedical and therapeutic properties [35] their natural roles [36], their production on inexpensive alternative substrates and their industrial potential, have been reviewed [28]. However their cost of production continues to remain very high. Using low-cost raw materials is a possible solution for this obstacle [28]. Another approach is to use renewable low cost starting materials from various sources including industrial wastes from frying oils, oil refinery wastes, molasses, starch rich wastes, cassava waste water and distilled grape marc [37, 38, 39]. These are explained in detail.

4.1 Agro-industrial wastes

These wastes are obtained at low cost from the respective processing industries and are as potent as low-cost substrates for industrial level biosurfactant production. Agricultural wastes such as rice water and water from the processing of cereals, pulse and molasses have potential to be used as excellent substrates for the production of biosurfactants.

P. aeruginosa can be cultivated in Cashew Apple Juice (CAJ) supplemented with peptone (5.0 g/L) and nutritive broth to obtain surfactants. Surface tension during the fermentation can be reduced by 41% when *P. aeruginosa* is cultivated in CAJ supplemented with peptone [40] compared to other amino acid sources. Several efforts have been undertaken in India to use some of the available agro-industrial wastes for biosurfactant production. Dubey and Juwarkar (41) studied biosurfactant production from

synthetic medium and industrial waste, viz. distillery, using an oily sludge isolate *P. aeruginosa* strain BS2. In synthetic medium separately supplemented with glucose and hexadecane as water-soluble and water-insoluble carbon sources, respectively, strain BS2 reduced the surface tension of the fermentation broth from 57 to 27 mN/m and produced biosurfactant to a yield of 0.97 g/L. Other cultures could utilize distillery wastes for their increased biosurfactant yield to 0.91 g/l.

4.1.1 Use of raw substrates

Vegetable oils and oil wastes

Frying oil is produced in large quantities for use both in the food industry and at the domestic scale. They can act as effective and inexpensive raw materials for biosurfactant production [42]. Similarly, several vegetable oils such as sunflower and soybean oils [43] have been used for the production of microbial surface agents. Oil wastes from vegetable oil refineries and the food industry have also been used as appropriate substrates for biosurfactant production. In addition, industrial oil wastes such as tallow, soap-stock, marine oils, lard and free fatty acids have a potential to induce microbial growth and lead to metabolite production. Waste oils generated from domestic uses, vegetable oil refineries or the soap industries also have been found to be suitable for biosurfactant production through microbial fermentation [44, 45, 46].

P. aeruginosa PACL strain, isolated from oil-contaminated soil taken from a lagoon has been grown in residual waste of soybean oils to produce biosurfactant by submerged fermentation in stirred tank reactors [26]. Sunflower seed oil and oleic acid can be used

for the production of rhamnolipids by *Thermus thermophilus* HB8. The potential production of rhamnolipids has been demonstrated using *Thermophilic eubacterium* [47]. Palm oil can be used for the simultaneous production of polyhydroxyalkanoates and rhamnolipids by *P. aeruginosa*. Production of rhamnolipids and L-(+)-rhamnose from rapeseed oil with *Pseudomonas* sp. DSM 2874 has also been reported [48]. Recently, the biosurfactant from *Candida glabrata* UCP1002 has been characterized and used for the removal of hydrophobic contaminants from soil. Vegetable fat wastes can be used as substrates [49] like other oil wastes and are easily and readily available in large quantities. Several plant-derived oils like jatropha oil, mesua oil, castor oils, ramtil oil and jojoba oil are not suitable for human consumption due to their unfavorable odor, color and toxic composition. Sunflower seed oil is directly hydrolyzed by secretion of lipase from the microbe and becomes a preferred carbon source for rhamnolipids production. *P. aeruginosa* 47 T2 can produce two main rhamnolipid homologs, (Rha-C10-C10) and (Rha-Rha- C10-C10), when grown in olive oil waste water or in waste frying oils obtained from olive/ sunflower (50:50; v/v), to produce as much as 8.1 g/L of rhamnolipids [50, 51, 52]. These are therefore easily available as alternate substrates. Incorporation of these cheaper oils and oil wastes in the industrial production media might potentially reduce the overall costs of biosurfactant production.

Olive oil mill waste effluent (OMWE)

Mediterranean countries produce more than 98% of the world's olive oil, which is estimated at over 2.5 million metric tons per year with about 75% being produced in the

European Union. The process of olive oil extraction results in a large amount of liquid waste. OMWE are characterized by an intensive dark brown color, a strong acidic smell and a high organic content (COD 220 g L^{-1}). OMWE is a black liquor and consists of a high content of organic matter (20-60 kg COD/m3), depending on the olive oil extraction procedure [68]. OMWE contains toxic substances such as polyphenols [69] making it unsuitable for human consumption in raw or processed form but has valuable organic substances such as sugars, nitrogen compounds, organic acids and residual oils which aid microbial growth. The large diversity of components found in OMWE (carbohydrates, polysaccharides, sugars, lipids and phenolic compounds) makes their treatment difficult, and their disposal becomes a critical environmental problem [70]. Thus, utilization of these materials is important from both environmental and economical points of view and can be considered not only as a waste to be treated but also a resource to be recovered.

The use of fungi can lead to OMWE valorization through the enzymes production [71]. The anaerobic treatment of OMWE can also represent an effluent recovery and methane production. Yeasts can also be used to degrade the phenolic compounds in OMWE. Specifically, *Yarrowia lipolytica* strains are good candidates for the OMWE treatment and recovery [72].

Enzymatic production of biosurfactants

Polyglycerol and carbohydrate fatty acid esters are broadly used as industrial detergents and as emulsifiers in a huge range of food formulations. Adelhorst *et al*. [53] have carried solvent-free esterification of simple alkyl-glycosides by means of molten fatty acids and

immobilized *C. antarctica* lipase. Fregapane *et al*. [54] obtained mono- and diesters of monosaccharides in elevated yields, with sugar acetyls as preliminary resource.

Lipase from *A. terreus* synthesizes a biosurfactant by transesterification involving natural oils and sugar alcohols [55]. Lipases may furthermore substitute phospholipases in the production of lysophospholipids. *Mucor miehei* lipase has been used for the transesterification of phospholipid in a variety of primary- and secondary alcohols [56]. Lipases may also be valuable in the synthesis of an entire range of amphoteric bio-degradable surfactants, specifically amino acid-based esters and amides.

Starchy substrates

Starchy waste materials are inexpensive raw materials suitable for the production of surface active agents. A major source of inexpensive starchy substrate is the potato processing industry. Potatoes are generally composed of 80% water, 17% carbohydrates, 2% protein, 0.1% fat and 0.9% vitamins, inorganic minerals and trace elements. They are a rich source of carbon (in the form of starch and sugars), nitrogen and sulfur (from protein), inorganic minerals, trace elements and vitamins. Thompson [57] reported the use of high solids (HS) and low solids (LS) potato effluents as substrates for surfactin production. He used effluents diluted 1:10, unamended and amended with trace minerals or corn steep liquor. *B. subtilis* 21332 grew on all three potato substrates regardless of addition of exogenous nutrients. Growth rate was higher in all HS- and LS-based media than in the B-PS (biotic purified starch) control. Potato process effluents (wastes from potato processing industries) can also be used to produce biosurfactant by *B. subtilis* [58].

Cassava wastewater is another carbohydrate-rich residue generated in large amounts during the preparation of cassava flour and is an attractive alternative substrate in fermentation processes. It has been used for surfactin production by *B. subtilis* [37]. Siddhartha *et al*. [59] used Cassava wastewater as a substrate for the simultaneous production of rhamnolipids and polyhydroxyalkanoates by *P. aeruginosa.*

4.2 Industrial wastes from animal and plant origin

Dairy Industry Whey

The whey from dairy industries is also a cheap and viable substrate for biosurfactant fermentation. About 6 liters of whey is produced per kg of paneer (cheese). A large portion of whey from the organized dairy sector is not utilized and is being disposed through the effluent treatment systems though it contains valuable nutrients (proteins, peptides, amino acids, lipids, minerals and vitamins).Thus, the effluent from the dairy industry, known as dairy wastewater supports good microbial growth and can be used for biosurfactant production [34, 59]. Daniel *et al*. [60] used dairy wastes as substrates and achieved production of high concentrations of sophorolipids using two-stage cultivation process for the yeast *Cryptococcus curvatus* ATCC 20509.

Animal fat

Animal fat and tallow can be obtained in large quantities from meat processing industries and have been used as a cooking medium for foods. Deshpande and Daniels [67] used animal fat for the production of sophorolipids biosurfactant production using the yeast, *C. bombicol*a. When fat was provided as the sole carbon source, the growth was poor.

However, a mixture of 10% glucose and 10% fat gave the highest level of growth indicating the requirement of an additional carbon source in the medium.

Molasses

This is a co-product of sugar industry generated during sugar manufacturing from either sugarcane or sugar beet and is a rich source of available carbon. Average values for the constituents of cane molasses (75% dry matter) are: 48-56% (total sugar), 9-12% (organic matter excluding sugar), 2.5% (protein), 1.5-5.0% (Potassium) 0.4-0.8% (Calcium), 0.06% (Magnesium), 0.06-2.0% (Phosphorus), 1.0-3.0 mg/kg (biotin), 15-55 mg/kg (pantothenic acid), 2,500-6,000 mg/kg (inositol) and 1.8 mg/kg (thiamine).

Patel and Desai [61] used molasses and corn-steep liquor as the primary carbon and nitrogen source to produce rhamnolipid biosurfactant using *P. aeruginosa* (Strain GS3). The biosurfactant production reached a maximum when a combination of 7% (v/v) molasses and 0.5% (v/v) corn-steep liquor waste used.

Soy Molasses

Soy molasses is a cheap feedstock as it is a low-value co-product of soybean processing and also rich in potentially fermentable sugar content and other growth factors useful for sustaining microbial growth. As health-conscious consumers continue to drive up the demands for soy protein-based foods and drinks, the soy protein industry has experienced a sustained 10% annual growth for the past several years with a market value of nearly $4 billion [62]. Thus, an increasing amount of agricultural wastes from soy cultivation is becoming available as a raw material for utilization in biosurfactant production. The major

components of the soluble carbohydrates in soy molasses are sucrose, raffinose and stachyose. Investigators have demonstrated that soy molasses could be used in fermentation processes to produce industrial chemicals such as lactic acid, butanol [63], sophorolipids biosurfactant [64] and poly-hydroxyalkanoates [65]. Daniel *et al*. [60] formulated a soy molasses-based medium for reduced cost production of sophorolipids biosurfactant by *C . bombicola.*

4.3 Other industrial wastes

Soap stock

Soap stock is a gummy, amber colored by-product of oilseed processing produced when hexane and other chemicals are used to extract and refine edible oil from the oilseeds. It has been used to produce emulsan and bio-dispersan. Emulsan forms and stabilizes oil-in-water emulsion, whereas bio-dispersan disperses the large solid limestone granules, forming micrometer-size water suspension [66]. *P. aeruginosa* strain LBI, isolated from petroleum contaminated soil, could produce surface-active rhamnolipids biosurfactant (RLLBI) by batch fermentation in a mineral salts medium with soapstock as the sole carbon source [44]. Biosurfactant production increased after nitrogen depletion and the maximum rhamnolipids concentration was 15.9 g/l. RLLBI produced stable emulsions with hydrocarbons (crude oil, kerosene, toluene, *n*-alkanes (C12-C14) and mineral oil) and vegetable oils (linseed oil, almond oil).

5. Microbial bioprocess development: maximal production and recovery

Development of bioprocesses is another important aspect of biosurfactant production using waste products. Several different issues need to be looked into before a standard procedure is laid out for setting up the process at industrial levels. These are described below.

5.1 Bio-process optimization

Type, quality and quantity of biosurfactant production is dependent on the cultural conditions *i.e.* pH, temperature, agitation, aeration, dilution rate, the concentration of metal ions, the nature of the carbon and nitrogen sources. There are lots of studies regarding biosurfactant production relating the optimization of their physicochemical properties [89]. Environmental factors are exceptionally significant in the yield and characteristics of the biosurfactant produced. In order to acquire large quantities of biosurfactant, it is essential to optimize the process conditions. An efficient and economic bioprocess is the bottleneck for any profit-making biotechnology industry. Several elements, media compositions and precursors affect the process of biosurfactant production. Different elements such as nitrogen, iron, and manganese affect the production of biosurfactants. Limitation of nitrogen enhances biosurfactant production in *P. aeruginosa* strain BS-2 [41] and *U. maydis* [73]. Addition of iron and manganese to the culture medium increased the production of biosurfactant by *B. subtilis*. The relative proportions of different elements to carbon in the reaction mix, such as C: N, C: P, C: Fe or C: Mg affects biosurfactant production [74]. The classical method of medium optimization involves changing one variable at a time, while keeping the others at fixed levels;

however, this method is time consuming and does not guarantee the optimal metabolite production. A statistical optimization strategy response surface methodology (RSM) has been developed for the optimization of process. Response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. This method could be used to determine the optimum media, inoculum and environmental conditions for the enhanced production of surfactin by *B. subtilis* [75]. RSM has also been applied to enhance biosurfactant production by *P. aeruginosa* AT10 [76], the probiotic bacterial strains *Lactococcus lactis* and *Streptococcus thermophilus* [77] and by *B. licheniformis* for the concomitant production of biosurfactants and protease RG1 using agro-products such as cornstarch and soy flour as carbon and nitrogen sources respectively. Such optimization methods would help the industry to design the best combination of cheaper substrates for media production and to use the most favorable environmental conditions for improved biosurfactant production. Current developments in the area of optimization of fermentation conditions have resulted in a considerable enhancement in production yields, making them more commercially attractive. Using the methods like experimental factorial design and response surface analysis, it is possible to conclude optimal operating circumstances to obtain a higher cellular growth, thus a higher cell-bound biosurfactant production yield. Optimization through factorial design and response surface analysis is a general practice in industrial biotechnology and numerous research workers have applied this technique for the optimization of cultural conditions [106, 107].

5.2 Influence of the Culture Medium Composition on Biosurfactant production

Biosurfactants are produced by a number of microorganisms, predominantly during their growth on water-immiscible substrates. However, some yeast may produce biosurfactants in the presence of different types of substrates, such as carbohydrates. The use of different carbon sources alters the structure of the biosurfactant produced and its properties and can be exploited to get products with desired properties for particular applications. There are a number of studies in biosurfactant production involving the optimization of their physicochemical properties [88, 89]. The composition and characteristics of biosurfactants are influenced by the nature of the nitrogen source as well as the presence of iron, magnesium, manganese, phosphorus and sulphur in the media.

5.2.1 Carbon Source

Till date, biosurfactants are unable to compete inexpensively with chemically synthesized compounds due to their high production costs and recovery system. These costs may be significantly reduced by the use of alternative sources of nutrients. Zinjarde and Pant (2002) demonstrated the surfactant biosynthesis by *Y. lipolytica* NCIM 3589 using soluble carbon source such as glucose, glycerol, sodium acetate. Sarubbo *et al.* 2001 [89] identified for the first time a biosurfactant produced by *Y. lipolytica* IA 1055 using glucose as carbon source and concluded that the induction of biosurfactant production is not dependent on the presence of hydrocarbons. Biosurfactant production by *B. subtilis* MTCC 2423 was monitored by measuring the reduction in surface tension of the cell-free broth.

Surface tension reduction was better when glucose, sucrose, tri sodium citrate, sodium pyruvate, yeast extract, and beef extract were used as carbon sources. Lactose has also been used as soluble substrate for the production of mannan-proteins by *Kluyveromyces marxianus* [90]. The maximum bioemulsifiers production was observed when the strain *C. glabrata* isolated from mangrove sediments was cultivated on cotton seed oil (7.5%) and glucose (5.0%), reaching values of 10 g L^{-1} after 144 hr. Kitamoto *et al*. [91] studied the production of mannosylerythritol lipids (MEL), a biosurfactant produced by *C. antarctica*, using different *n*-alkanes as carbon source. The productivity of MEL was significantly affected by the chain-length of the alkane substrates, with the highest productivity obtained from *n*-octadecane. Cavalero and Cooper, 2003 [92] have shown that the sophorolipid yield from *C. bombicola* ATCC 22214 increases with the *n*-alkane chain length (from C12 to C15). This indicated that different microbes respond differently to the carbon sources. The soy molasses, a byproduct from the production of soybean oil, plus oleic acid were tested as carbon sources for the production of sophorolipids by the yeast *C. bombicola* [64]. The purified SLs were obtained at 21 g l–1 and were 97% in lactone form. The surface properties of the SLs obtained from the soy molasses/oleic acid fermentation had minimum surface-tension values of 37 mN m^{-1} (pH 6) and 38 mN m^{-1} (pH 9), and critical micelle concentration values of 6 mg l^{-1} (pH 6) and 13 mg l^{-1} (pH 9).

The described C-sources, such as glucose, glycerol, acetates and other organic acids, as well as pure n-alkanes are quite expensive and cannot reduce the cost of

biosurfactant production. An approach to lessen the cost is partial or complete replacement of pure reagents with industrial/agricultural mixtures.

5.2.2 Nitrogen Source

Nitrogen is important in the biosurfactant production medium because it is an essential component of the proteins that are essential for the growth of microbes and for production of enzymes for the fermentation process. Several sources of nitrogen have been used for the production of biosurfactants, such as urea, peptone, ammonium sulphate, [103] ammonium nitrate, [94] sodium nitrate, [45] meat extract and malt extract [95]. Yeast extract is the most widely used nitrogen source for biosurfactant production, but its required concentration depends on the nature of microorganism and the culture medium to be used. The production of biosurfactants often occurs when the nitrogen source is depleted in the culture medium, during the stationary phase of cell growth.

During the production of biosurfactant by the yeast *R. glutinis* IIP30, the use of potassium nitrate gives better yields in comparison to other nitrogen sources such as ammonium sulphate or urea [96]. Lukondeh *et al.* [90] investigated the production of biosurfactant by *K. marxianus* FII 510700 using yeast extract (2 g L^{-1}) and ammonium sulphate (5 g L^{-1}) as nitrogen sources.

5.3 Environmental Factors Affecting the Production

Environmental factors are extremely important in the yield and characteristics of the biosurfactant produced. In order to obtain large quantities of biosurfactant it is necessary

to optimize the process conditions because the production of a biosurfactant is affected by variables such as pH, temperature, aeration and agitation speed.

5.3.1 pH

The effect of pH in the biosurfactant production by *C. antarctica* has been investigated using phosphate buffer with pH values varying from 4 to 8. All conditions used resulted in a reduction of biosurfactant yield when compared to distilled water [91]. Zinjarde and Pant [93] studied the influence of initial pH in the production of a biosurfactant by *Y. lipolytica*. The best production of biosurfactant occurs when the pH was 8.0, which is the natural pH of sea water. The acidity of the production medium was the parameter studied in the synthesis of glycolipids by *C. antarctica* and *C. apicola*. When pH is maintained at 5.5, the production of glycolipids reaches a maximum. The synthesis of the biosurfactant decreased without the pH control indicating the importance of maintaining it throughout the fermentation process [45].

5.3.2 Temperature

Most of the biosurfactant productions reported so far have been performed in a temperature range of 25 to 30˚C. Casas and Ochoa [97] noticed that the amount of sophorolipids obtained in the culture medium of *C. bombicola* at temperature of 25˚C or 30˚C is similar. Nevertheless, the fermentation performed at 25˚C presents a lower biomass growth and a higher glucose consumption rate in comparison to the fermentation performed at 30˚C. Desphande and Daniels [67] observed that the growth of *C. bombicola* reaches a maximum at a temperature of 30˚C while 27˚C is the best temperature for the

production of sophorolipids. In the culture of *C. antarctica*, temperature causes variations in the biosurfactant production. The highest mannosylerythritol lipids production was observed at 25˚C for the production with both growing and resting cells [91].

5.3.3 Metal ion concentration

Metal ions concentrations play a very important role in the production of some biosurfactants as they form important cofactors of many enzymes. The overproduction of surfactin biosurfactant occurs in presence of Fe^{2+} in mineral salt medium. The properties of surfactin are modified in the presence of inorganic cations such as overproduction [98].

5.3.4 Aeration and Agitation

N. erythropolis and *A. calcoaceticus* produce less biosurfactant due to the increase of shear stress but on the other hand biosurfactant production with yeasts generally increases with stirring and aeration rates [7]. Adamczak and Bednarsk [99] studied the influence of aeration in the biosurfactant synthesis by *C. antarctica* and observed that the best production (45.5 g l^{-1}) is obtained when air flow rate is 1 vvm and the dissolved oxygen concentration is maintained at 50% of saturation. Nevertheless, changing the air flow rate to 2 vvm, there is a high foam formation and the biosurfactant production decreases up to 84% [100]. Attempts have been made to reduce the end-product inhibition in surfactin production by isolating surfactin from the culture using foam separation and aqueous two-phase cultivation [101, 102].

5.4 Product recovery

Even if optimum production is obtained using optimal media and cultural conditions, the production process is still incomplete without an efficient and economical means for the downstream processing. For many microbiological products, the downstream processing costs account for 60% of the total production costs. Several methods have been developed for improving the recovery of Biosurfactants (Table-3).

Table-3: Methods for the recovery of biosurfactants

Sr. No	Method(s)	Mechanism(s)	Reference(s)
1.	Adsorption on wood	Adsorption	[78, 79]
2.	Adsorption on Polystyrene	Adsorption	[80]
3.	Ion exchange Chromatography	Charge separation	[80]
4.	Solvent extraction	Dissolves in organic solvents	[81]
5.	Centrifugation	Due to Centrifugal force	[46]
6.	Acid Precipitation	Insoluble at low pH	[75]
7.	Membrane Ultra filtration	Micelles formation	[82]
8.	Selective Crystallization	Redissolution in organic Solvents	[83]
9.	Ammonium Sulphate precipitation	Salting out of protein	[83]
10.	Organic Solvent extraction	Solubility in organic solvents	[79]
11.	Foam fractionation	Surface activity	[84]
12.	Thin layer chromatography	Difference in relative flow against solvent	[85]
13.	Dialysis	Difference in solute concentration	[86]
14.	Lyophilization	Cryodesiccation	[86]
15.	Iso-electric focusing	Electric charge difference	[86]

These procedures take advantage of some of the properties of biosurfactants such as their surface activity or their ability to form micelles and/or vesicles and are particularly applicable for large-scale continuous recovery of extracellular biosurfactants from culture

broth. A few examples of such biosurfactant recovery strategies include foam fractionation [84], ultra-filtration [87], adsorption-desorption on polystyrene resins and ion exchange chromatography [80], and adsorption-desorption on wood-based activated carbon (WAC) [79] Ammonium sulphate precipitation and selective crystallization [83]. Cheap and less toxic solvents such as methyl tertiary-butyl ether (MTBE) have been successfully used in recent years to recover biosurfactants produced by *Rhodococcus* [81]. These types of low cost, less toxic and highly available solvents can be used to cut the recovery expenses substantially and minimize the environmental hazards.

5.5 Metabolic characterization of biosurfactants

Microbial production of biosurfactant is growth associated. Growth-associated biosurfactant production has been reported for the release of biodispersan by *A. calcoaceticus* [104]. In addition, biosurfactant production may possibly occur (or be stimulated) by growing the microbial cells below growth restrictive conditions. *P. aeruginosa* shows an over production of rhamnolipid when the culture reaches the stationary growth phase due to limitation of the nitrogen source. Velraeds *et al.* showed that biosurfactant release by lactobacilli is optimal for cell in the stationary phase [105]. Additionally, a direct relation exists between biosurfactant production and cell growth along the fermentation process.

Conclusions

This present review provides basic scientific information on biosurfactants that is required to harness natural processes and develop methods to accelerate these processes for

economically viable production of biosurfactants. Despite the advantages of biosurfactant synthesis, its industrial use is still limited due to the high costs involved in the production process. The economics of biosurfactant production may be significantly impacted through use of inexpensive carbon substrates. In this review, we have presented a thorough investigation of various means of making biosurfactants economical. Rapid advances in the last few years helped us to understand the process of biosurfactant fermentation by many microorganisms. The use of culture independent molecular techniques has definitely helped us to understand the microbial community dynamics, structure and assisted in providing the insight in to details of biosurfactant production which facilitated to make the technology safer and reliable. With the exciting new development in this field and focus on interdisciplinary research combined with technologies of large-scale fermentation and genetic and metabolic engineering, biosurfactants will be commercially successful compounds of the future.

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