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Biosurfactants production from cheese whey

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Abstract

Biosurfactants are molecules that exhibit pronounced surface and emulsifying activities, produced by a variety of microorganisms. A host of interesting features of biosurfactants, such as higher biodegradability, lower toxicity, and effectiveness at extremes of temperature, pH and salinity; have led to a wide range of potential applications in the fields of oil recovery, environmental bioremediation, food processing and medicine. In spite of the immense potential of biosurfactants, their use still remains limited, mainly due to their high production and extraction costs, low yields in production processes

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and lack of information on their toxicity towards human systems. However, the use of cheaper substrates and optimal growth and production conditions coupled with novel and efficient multistep downstream processing methods and the use of recombinant and mutant hyper producing microbial strains can make biosurfactant production economically feasible. Often, the amount and type of a raw material can contribute considerably to the production cost; it is estimated that raw materials account for 10 to 30% of the total production costs in most biotechnological processes. Thus, to reduce this cost it is desirable to use low-cost raw materials. One possibility explored extensively is the use of cheap and agro-based raw materials as substrates for biosurfactant production. A variety of cheap raw materials, including plant-derived oils, oil wastes, starchy substances, cheese whey and distillery wastes have been reported to support biosurfactant production. Future biosurfactant research should, therefore, be more focused on the economics of biosurfactant production processes, particularly through the use of alternative low-cost fermentative media. This review looks at the future perspectives of large-scale profitable production of biosurfactants.

Introduction

Biosurfactants are molecules that exhibit pronounced surface and emulsifying activities, produced by a variety of microorganisms. A wide range of chemical structures can be found among these compounds, such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids [1-7]. Hence, it is reasonable to expect diverse properties and physiological functions for different groups of biosurfactants. Comparing with chemical surfactants, these compounds have several advantages such as lower toxicity, higher biodegradability and effectiveness at extreme temperatures or pH values [8, 9]. Moreover, biosurfactants can be tailor-made to suit different applications by changing its production conditions [10, 11]. Although most biosurfactants are considered to be secondary metabolites, some may play essential roles for the survival of biosurfactant-producing microorganisms through facilitating nutrient transport or microbe–host interactions or by acting as biocide agents. Its roles include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing and biofilm formation [7, 12-15].

Biosurfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phases that have different degrees of polarity and hydrogen bonding, such as oil and water or air and water interfaces. In addition to this behaviour, their diversity, environmentally friendly nature, suitability for large-scale production and selectivity, has driven most of the research in biosurfactants

field for environmental applications [16-19]. Legal aspects such as stricter regulations concerning environmental pollution by industrial activities and health regulations will also strongly influence the chances of biodegradable biosurfactants replacing their chemical counterparts [10].

In spite of the immense potential of biosurfactants, their use still remains limited, mainly due to their high production and extraction costs, low yields in production processes and lack of information on their toxicity towards human systems. However, the use of cheaper substrates and optimal growth and production conditions coupled with novel and efficient multistep downstream processing methods and the use of recombinant and mutant hyper producing microbial strains can make biosurfactant production economically feasible [15, 20-28]. Often, the amount and type of a raw material can contribute considerably to the production cost; it is estimated that raw materials account for 10 to 30% of the total production costs in most biotechnological processes [21, 29, 30]. Thus, to reduce this cost it is desirable to use low-cost raw materials. One possibility explored extensively is the use of cheap and agro-based raw materials as substrates for biosurfactant production. A variety of cheap raw materials, including plant-derived oils, oil wastes, starchy substances, cheese whey and distillery wastes have been reported to support biosurfactant production [8, 20, 21, 31-37]. Future biosurfactant research should, therefore, be more focused on the economics of biosurfactant production processes, particularly through the use of alternative low-cost fermentative media.

This review compiles studies on the optimization of biosurfactant production based on cheaper raw materials and looks at its future perspectives of large-scale profitable production.

Synthetic surfactants and biosurfactants

A surfactant molecule is amphiphilic which means it contains a hydrophilic and a hydrophobic domain. Frequently, the non-polar hydrophobic domain is a hydrocarbon chain, while the polar part appears in many variations [19]. It is possible to find both non-ionic (ethoxylates, ethylene and propylene oxide copolymers, among others) and ionic (fatty acids, ester sulphonates or sulphates (anionic) and quaternary ammonium salts (cationic)) commercially available surfactants [13, 15, 38]. Microbial compounds with high surface and emulsifying activities are named biosurfactants. These molecules possess a broad range of different chemical structures and, although there are many biosurfactant-producing microorganisms, they are mainly produced by hydrocarbon utilizing microorganisms. This diversity of chemical structures constitutes one of the major advantages of the biosurfactants as compared with the synthetic ones, as there are some structural types of surfactants produced using biological systems with interesting features and applications that cannot easily be synthesized by chemical processes [39]. Moreover, it is possible to

Table 1. Biosurfactants produced by microorganisms (adapted from [19, 38, 44])

Class	Biosurfactant	Microorganisms	References
Low molecular weight	Rhamnolipids	<i>Pseudomonas aeruginosa</i> , <i>Serratia rubidea</i>	[43]
	Trehalose lipids	<i>Arthrobacter paraffineus</i> , <i>Rhodococcus erythropolis</i> , <i>Mycobacterium</i>	[45, 46]
	Sophorose lipids	<i>Candida lipolytica</i> , <i>Torulopsis bombicola</i>	[47]
	Cellobiose lipids	<i>Ustilago maydis</i>	[10]
	Viscosin	<i>Pseudomonas fluorescens</i>	[48]
	Surfactin	<i>Bacillus subtilis</i> , <i>Bacillus pumilus</i>	[49]
	Polymixins	<i>Bacillus polymyxa</i>	[50]
	Phospholipids	<i>Acinetobacter</i> , <i>Thiobacillus thiooxidans</i>	[51]
	Flavolipids	<i>Flavobacterium sp.</i>	[52]
	Lipopeptides	<i>Bacillus subtilis</i> (Iturin A), <i>Bacillus pumilus</i> , <i>Bacillus licheniformis</i> , <i>Pseudomonas syringae</i> , <i>Pseudomonas fluorescens</i>	[53-56]
	Serrawettin	<i>Serratia marcescens</i>	[57]
	Fatty acids	<i>Nocardia erythropolis</i> , <i>Arthrobacter paraffineus</i> , <i>Corynebacterium lepus</i> , <i>Penicillium spiculispurum</i> , <i>Talaromyces trachyspermus</i>	[21]
	Glycolipid	<i>Tsukamurella sp.</i>	[58]
	Sulfonolipids	<i>Capnocytophaga</i> , <i>Corynebacterium</i>	[59]
	Diglycosyl diglycerides	<i>Rhizobium trifolii</i>	[60]
	Glicolipid	<i>Streptococcus thermophilus A</i>	[61]
Glicoprotein	<i>Lactococcus lactis 53</i>	[62]	
Mannosylerythritol lipids	<i>Candida antarctica</i>	[63]	
High molecular weight	Alasan	<i>Acinetobacter radioresistens</i>	[64]
	Emulsan	<i>Acinetobacter calcoaceticus</i>	[65]
	Biodispersan	<i>Acinetobacter calcoaceticus</i>	[66]
	Liposan	<i>Candida lipolytica</i>	[67]
	Sulfated polysaccharide	<i>Halomonas eurihalina</i>	[68]
	N-acetyl and O-pyruvil heteropolysaccharide	<i>Pseudomonas fluorescens</i>	[69]

design biosurfactants to suit different applications by changing the microorganisms growth substrates and/or conditions [11, 38]. In addition, comparing with chemical surfactants, these compounds present a lower toxicity [15, 18], a higher biodegradability [15, 17] and effectiveness at extreme temperatures or pH values, which make them interesting molecules to use in environmental applications.

Biosurfactants production is relatively simple and inexpensive when alternative processes and substrates are used [4, 5, 8, 11, 13, 15, 21, 33, 38, 40, 42]. There are many potentially useful biosurfactants, including both ionic and non-ionic surfactants which range from short fatty acids to large polymers (Table 1).

Applications of biosurfactants

During the last 2-3 decades a wide variety of microorganisms have been reported to produce numerous types of biosurfactants [19]. Biosurfactants have many potential applications including enhanced oil recovery, crude oil drilling lubricants, surfactant-aided bioremediation of water-insoluble pollutants, and in the health care and food processing industries. Other developing areas of biosurfactant use are in cosmetic and soap formulations, foods, and dermal as well as transdermal drug delivery systems as reflected in Japanese patent literature [21]. Several reviews in the past decade have summarized the possible roles of biosurfactants [7, 12, 13, 15, 40, 44]. Undoubtedly, the largest possible market for biosurfactant is the oil industry, both for petroleum production and for incorporation into oil formulations [70].

Other applications related to the oil industries include oil spill bioremediation/dispersion, both inland and at sea, removal/mobilization of oil sludge from storage tanks and enhanced oil recovery [17, 71-73]. In addition, there is other interesting market for biosurfactants that includes emulsion polymerization for paints, paper coatings and industrial coatings [74]. Moreover, biosurfactants exhibit a variety of useful properties for the food industry specially as emulsifiers, foaming, wetting, solubilizers [19], anti-adhesive and antimicrobial agents [7, 12, 61, 62, 75, 76]. Table 2 summarizes some of the industrial applications of chemical surfactants and biosurfactants.

It has also been reported that biosurfactants have potent antimicrobial applications including antifungal, antibacterial, antimycoplasmal and antiviral activities [7]. Therefore, medically relevant uses of biosurfactants include their role as anti-adhesive agents to pathogens, making them useful for treating many diseases and as therapeutic and probiotic agents [77-80]. Biosurfactants have been used for gene transfection, as ligands for binding immunoglobulins, as adjuvants for antigens and also as inhibitors for fibrin clot formation and activators of fibrin clot lysis [7]. Examples of biosurfactants applications in the medical field are given in Table 3. Furthermore, biosurfactants have the

Table 2. Industrial applications of chemical surfactants and biosurfactants (adapted from [13]).

Industry	Application	Role of Surfactants
Petroleum	Enhanced oil recovery	Improving oil drainage into well bore; stimulating release of oil entrapped by capillaries; wetting of solid surfaces; reduction of oil viscosity and oil pour point; lowering of interfacial tension; dissolving of oil
	De-emulsification	De-emulsification of oil emulsions; oil solubilisation; viscosity reduction, wetting agent
Environmental	Bioremediation	Emulsification of hydrocarbons; lowering of interfacial tension; metal sequestration
	Soil remediation and flushing	Emulsification through adherence to hydrocarbons; dispersion; foaming agent; detergent; soil flushing
Food	Emulsification and de-emulsification	Emulsifier; solubiliser; demulsifier; suspension, wetting, foaming, de-foaming, thickener, lubricating agent
	Functional ingredient	Interaction with lipids, proteins and carbohydrates, protecting agent
Biological and Medical	Microbiological	Physiological behaviour such as cell mobility, cell communication, nutrient accession, cell-cell competition, plant and animal pathogenesis
	Pharmaceuticals and therapeutics	Antibacterial, antifungal, antiviral agents; adhesive agents; immunomodulatory molecules; vaccines; gene therapy
Agricultural	Biocontrol	Facilitation of biocontrol mechanisms of microbes such as parasitism, antibiosis, competition, induced systemic resistance and hypovirulence
Bioprocessing	Downstream processing	Biocatalysis in aqueous two-phase systems and microemulsions; biotransformations; recovery of intracellular products; enhanced production of extracellular enzymes and fermentation products
Cosmetic	Health and beauty products	Emulsifiers, foaming agents, solubilisers, wetting agents, cleansers, antimicrobial agent, mediators of enzyme action

Table 3. Examples of biosurfactants application in the medical field (adapted from [7]).

Microorganism	Biosurfactant type	Activity/Application	Reference
<i>Pseudomonas aeruginosa</i>	Rhamnolipid	<ul style="list-style-type: none"> antimicrobial activity against <i>Mycobacterium tuberculosis</i> anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses 	[42, 76, 81, 82]
<i>Bacillus subtilis</i>	Surfactin	<ul style="list-style-type: none"> antimicrobial and antifungal activities inhibition of fibrin clot formation hemolysis and formation of ion channels in lipid membranes antitumor activity against Ehrlich's ascite carcinoma cells antiviral activity against human immunodeficiency virus 1 (HIV-1) 	[83-88]
<i>Bacillus subtilis</i>	Pumilacidin (surfactin analog)	<ul style="list-style-type: none"> antiviral activity against herpes simplex virus 1 (HSV-1) inhibitory activity against H⁺, K⁺-ATPase and protection against gastric ulcers <i>in vivo</i>. 	[89]
<i>Bacillus subtilis</i>	Iturin	<ul style="list-style-type: none"> antimicrobial activity and antifungal activity against profound mycosis effect on the morphology and membrane structure of yeast cells increase in the electrical conductance of biomolecular lipid membranes nontoxic and nonpyrogenic immunological adjuvant 	[1, 90-93]
<i>Bacillus licheniformis</i>	Lichenysin	<ul style="list-style-type: none"> antibacterial activity chelating properties that might explain the membrane disrupting effect of lipopeptides 	[94-97]
<i>Candida antartica</i>	Mannosylerythritol lipids	<ul style="list-style-type: none"> antimicrobial, immunological and neurological properties induction of cell differentiation in the human promyelocytic leukemia cell line HL60 induction of neuronal differentiation in PC12 cells 	[63, 98-103]
<i>Rodococcus erythropolis</i>	Trehalose lipid	<ul style="list-style-type: none"> antiviral activity against HSV and influenza virus 	[45, 46]
<i>Streptococcus thermophilus</i>	Glycolipid	<ul style="list-style-type: none"> anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses 	[75, 80, 104-106]
<i>Streptococcus mitis</i>	Not identified	<ul style="list-style-type: none"> anti-adhesive activity against <i>Streptococcus mutans</i> 	[107, 108]
<i>Lactobacillus</i>	Surlactin	<ul style="list-style-type: none"> anti-adhesive activity against several pathogens including enteric bacteria 	[109-113]
<i>Lactococcus lactis</i>	Not identified	<ul style="list-style-type: none"> anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses 	[79, 80]

potential to be used as anti-adhesive biological coatings for medical insertional materials, thus reducing hospital infections and use of synthetic drugs and chemicals [77]. They may also be incorporated into probiotic preparations to combat urogenital tract infections and pulmonary immunotherapy [7]. However, in spite of the immense potential of biosurfactants in the medical field, their use still remains limited, possibly due to their high production and extraction cost and lack of information on their toxicity towards human systems.

The economics of biosurfactant production

As discussed above many of the potential applications that have been considered for biosurfactants depend on whether they can be produced economically; therefore, much effort in process optimization and at the engineering and biological levels has been carried out. The success of biosurfactant production depends on the development of cheaper processes and the use of low cost raw materials [9, 29, 30, 40]. In fact, as with any other biotechnological process the economy represents the bottleneck of the process. Nevertheless, currently many classical industries are being redirected towards emerging technologies, namely biotechnology. According to the European Commission data the industrial biotechnology world market is expected to go beyond M€ 130 000 by 2010.

Surfactants constitute an important class of industrial chemicals widely used in almost every sector of modern industry. According to Technical Insights, a division of Frost & Sullivan, microbial surfactants have begun to enjoy a market [114]. Hester [115] of the same company estimated that biosurfactants could capture 10% of the surfactant market by the year 2010 with sales of \$200 million US [115]. They have found that the most promising applications are oil spill and oil-contaminated tanker cleanup, removal of crude oil from sludge, enhanced oil recovery, bioremediation of sites contaminated with hydrocarbons, other organic pollutants and heavy metals. At the moment, most of the commercially available surfactants are chemical surfactants, mainly petroleum-derived. The chance of biosurfactants replacing their chemical counterparts is mainly related with the cost, functionality and production capacity to meet the need of the intended application. It can be accepted a high production cost for a biosurfactant if it is a high value product and/or the production volumes are low, such as for medicines for example. However, for the most common biosurfactant applications, namely environmental ones, the high volumes required make high production costs unbearable. Therefore, research efforts must focus on the development of processes of biosurfactant production with reduced costs. Some of the factors that can influence the costs are the selected or engineered microorganisms; the developed process; the choice of the growth substrate; the process by-products and product recovery. One of the most important factors to consider when

developing a biosurfactant production process is the downstream processing required for product recovery. The downstream techniques are often costly and therefore, whenever possible, simple and inexpensive techniques (for example gravity separation) should be chosen. The most common biosurfactant recovery methods are either extraction with solvents (e.g. chloroform-methanol, dichloromethane-methanol, butanol, ethyl acetate, pentane, hexane, acetic acid, ether) or acid precipitation. However, there are some studies on the use of ammonium sulfate precipitation, crystallization centrifugation, adsorption and foam fractionation, among others [4, 8].

Table 4. Use of inexpensive raw materials for the production of biosurfactants by several microorganisms (adapted from [15])

Low cost or waste raw material	Biosurfactant type	Microbial strain	Reference
Rapeseed oil	Rhamnolipids	<i>Pseudomonas</i> species DSM 2874	[116]
Babassu oil	Sophorolipids	<i>Candida lipolytica</i> IA 1055	[117]
Turkish corn oil	Sophorolipids	<i>Candida bombicola</i> ATCC 22214	[118]
Sunflower and soybean oil	Rhamnolipid	<i>Pseudomonas aeruginosa</i> DS 10-129	[25]
Sunflower oil	Lipopeptide	<i>Serratia marcescens</i>	[119]
Soybean oil	Mannosylerythritol lipid	<i>Candida</i> sp. SY16	[120]
Waste frying oils	Rhamnolipid	<i>Pseudomonas aeruginosa</i> 47T2 NCBI 40044	[121]
Soybean soapstock waste	Rhamnolipid	<i>Pseudomonas aeruginosa</i> LBI	[122]
Sunflower oil soapstock waste	Rhamnolipid	<i>Pseudomonas aeruginosa</i> LBI	[43, 123]
Oil refinery wastes	Glycolipids	<i>Candida antarctica</i> and/or <i>Candida apicola</i>	[124]
Soybean oil refinery wastes	Rhamnolipids	<i>Pseudomonas aeruginosa</i> AT10	[125]
Curd whey and distillery wastes	Rhamnolipid	<i>Pseudomonas aeruginosa</i> strain BS2	[126, 127]
Potato process effluents	Lipopeptide	<i>Bacillus subtilis</i>	[128-131]
Cassava flour wastewater	Lipopeptide	<i>Bacillus subtilis</i> LB5a	[132-134]
Cheese whey	Glicolipid or Glicoprotein	<i>Streptococcus thermophilus</i> A or <i>Lactococcus lactis</i> 53	[20, 78]

Moreover, as discussed previously, to reduce the production costs it is desirable to use low-cost raw materials. Table 4 summarizes some examples on the use of inexpensive raw materials for the production of biosurfactants by various microbial strains. Several studies on the use of agro-based crops (cassava, soybean, sugar beet, sweet potato, potato, and sweet sorghum) and crop residues (bran and straw of wheat and rice; bagasse of sugarcane and cassava; residues from the coffee processing industry; residues of the fruit processing; waste from oil processing mills; and others such as carob pods, tea waste, chicory roots) have been reported [20, 21, 31-37, 78, 135]. Additional substrates, such as molasses, whey milk or distillery wastes have also been suggested as alternative raw materials for the biosurfactants production [136, 137].

Biosurfactants production

Owing to the large surface-to-volume ratio and diverse biosynthetic capabilities, microorganisms are promising candidates in the search for enlarging our present range of surfactants. Major classes of biosurfactants include glycolipids, phospholipids and fatty acids, lipopeptides/lipoproteins, polymeric surfactants and particulate surfactants [138].

Depending upon the nature of the biosurfactant and the producing microorganisms, the following patterns of biosurfactant production by fermentation are possible: (a) growth-associated production, (b) production under growth limiting conditions, (c) production by resting/nongrowing cells, and (d) production associated with the precursor augmentation. In the case of growth-associated biosurfactant production, there exists a parallel relationship between the substrate utilization, growth and biosurfactant production [138]. Biosurfactants are usually secondary metabolites and most of them are produced on hydrocarbons; however some can also be produced on carbohydrates. Production is most often growth-associated. In this case, they can either use the emulsification of the substrate (extracellular) or facilitate the passage of the substrate through the membrane (cell wall associated).

Cell growth and the accumulation of metabolic products are strongly influenced by medium compositions such as carbon sources, nitrogen sources, growth factors, and inorganic salts. Thus, it is difficult to search for the major factors and to optimize them for biotechnological processes as several parameters are involved [139]. Environmental factors and growth conditions such as pH, temperature, agitation, and oxygen availability also affect biosurfactant production through their effects on cellular growth or activity [4].

All microorganisms require for growth a source of carbon, hydrogen, nitrogen, oxygen, and, to a smaller degree, sulphur and phosphorus [140]. These materials are available in many forms. The choice of raw materials is very important to the overall economics of the process. Particularly in the bulk

product market, production costs are influenced by the price of the feedstocks and other raw materials. Availability, stability, and variability of each component must also be considered. The amount to be used, form (solid or liquid), packaging, transportation, purity, and production as a by-product of a process are all factors influencing raw materials costs. Lower purity materials are less expensive and can be tolerated in most cases.

In particular, the carbon source is very important in biosurfactant production and a wide variety has been used. They include hydrocarbon, carbohydrate, and vegetable oil sources. Some organisms produce biosurfactants only in hydrocarbons, others only in carbohydrates, and still others use several substrates, in combination or separately.

Biosurfactant production using oils and oil wastes

World production of oils and fats is about 2.5-3 million tons, being 75% derived from plants [121]. Several researchers reported the use of plant-derived oils as effective and cheap raw materials for biosurfactant production, namely rapeseed oil [116], Babassu oil, Canola oil and corn oil [37, 117, 118]. Likewise, vegetable oils such as sunflower and soybean oils [107, 119, 120] were used for the production of rhamnolipid, sophorolipid and mannosylerythritol lipid biosurfactants by a variety of microorganisms. Apart from various vegetable oils, oil wastes from vegetable oil refineries and the food industry were also reported as good substrates for biosurfactant production [44, 121-125]. In addition, industrial oil wastes such as tallow, soapstock, marine oils, lard and free fatty acids can potentially induce microbial growth and metabolite production owing to their typical fatty acid composition. These oils and oil wastes are readily available in good amounts throughout the world. Additionally, as waste disposal is a growing problem, an increasing interest in its use in microbial transformation has been observed over the years. Nevertheless, the oils used to date for biosurfactant production are mostly edible oils and expensive. A range of alternative plant-derived oils not suitable for human consumption, such as jatropha oil, mesua oil, castor oils, ramtil oil and jojoba oil, are available at much cheaper rates. Incorporation of these cheaper oils and oil wastes in the industrial production of biosurfactants could represent a significant reduction of the overall production costs [15].

The use of domestic vegetable oils for the production of biosurfactants by *Tsukamurella spec* DSM 44370 was reported by Vollbrecht and co-workers [141]. Several vegetable oils were tested and the best results were achieved with oleic acid-rich and rapeseed oils (C 22:1). The physiochemical characterization of the biosurfactants produced under these conditions showed reductions of the water surface tension of 37 mN/m for (GL1), and of 49 mN/m for glycolipids GL2 and GL3. The glycolipids produced by *Tsukamurella* were found to be as effective as commercially available surfactants. Another example

of the use of oils as raw materials for biosurfactants production was described by Sarubbo and co-workers [142]. In their work they evaluated the production of bioemulsifiers by two strains of *Candida lipolytica* (1055 and 1120) using media supplemented with 5% BabaCu oil and 1% glucose as carbon source. These bioemulsifiers were found to be produced as secondary metabolites at the end of the exponential growth phase and beginning of the stationary growth phase. The same authors also studied the co-utilization of Canola oil and glucose on the production of the bioemulsifiers by the same yeast strain [37]. Moreover, it was reported the use of olive oil mill effluent (OOME) as a new substrate for biosurfactant production (rhamnolipids) by *Pseudomonas sp.* JAMM [143]. This oil effluent is a relevant pollutant of the agricultural industry especially in the Mediterranean countries. This effluent is a black liquor containing the water-soluble fraction of ripe olives and water that is used in the process of olive oil extraction. Several strains were screened for growth in this medium and it was found that strains belonging to the genus *Pseudomonas* were good biosurfactant-producing candidates. Additionally, the same authors studied the biosurfactant production by *Pseudomonas* strain 42A2 using a sub-product from the distillation of non-specific mixtures of vegetable oils with a high content of oleic acid (98% w/w) as raw material. This biosurfactant has been characterized as being 7, 10 dihydroxy-8E-octadecanoic acid [144]. Moreover, Abalos and co-workers [125] have reported the use of soybean oil refinery wastes for the production of new rhamnolipids by *P. aeruginosa* AT110.

Furthermore, *Candida antarctica* and *Candida apicola* were found to produce glycolipids when grown in a cultivation medium supplemented with oil refinery waste, either with soapstock (5-12% v/v) or post-refinery fatty acids (from 2 to 5% v/v). The efficiency of glycolipids synthesis was from 7.3 to 13.4 g/L and from 6.6 to 10.5 g/L in the medium supplemented with soapstock and post-refinery fatty acids, respectively [124]. Nitschke et al. [122] also evaluated edible oil soapstocks as alternative low-cost substrates for the production of rhamnolipids by *P. aeruginosa* LBI strain. Wastes obtained from soybean, cottonseed, babassu, palm and corn oil refinery were tested. The soybean soapstock waste was the best substrate, generating 11.7 g/L of rhamnolipids and a production yield of 75%. Vegetable oils and residues from vegetable oil refinery are among the most used low-cost substrates for rhamnolipids production [122].

Frying oil is produced in large quantities for use both in the food industry and at the domestic scale. After being used, cooking oil changes its composition and contains more than 30% of polar compounds [145] depending on the variety of food, the type of frying and the number of times it has been used. Haba and co-workers [121] compared the composition of used olive and sunflower oils with the standard unused oils in their study and found that the

most important difference in the used oil is the presence of 22.52% of fatty acids of low chain length (<C10), myristic acid and lauric acid. In their study they screened 36 microorganisms for production of biosurfactants in submerged culture with 2% waste olive or sunflower oil as carbon source using the lowering of surface tension (below 40 mN/m) as the selection criteria. After 72 h of growth most of the *Pseudomonas* strains tested showed satisfactory growth when cultivated on used olive or used sunflower oil. Used olive oil was found to be a better substrate for cell growth, as well as for biosurfactant production. The surface tension was lowered below 35 mN/m for most of the *Pseudomonas* strains tested and the biosurfactants produced were rhamnolipids. Results achieved for the production of lipopeptides by various *Bacillus* strains were not as good as the ones achieved with *Pseudomonas*. In addition, the other strains tested (*Rhodococcus* sp., *Acinetobacter calcoaceticus* and *Candida* sp.) did not compare favourably with the previous ones. Waste or used lubricating (lube) oils have become a serious environmental problem. In the environment, the waste oil can bind to organic matter, mineral particles and organisms [146]. Mercade and co-workers [147] reported the screening and selection of microorganisms capable of utilizing waste lube oil for producing biosurfactants. In their study they isolated 44 different microorganisms from contaminated soil samples but only 10% of these strains produced biosurfactants. Further characterization of these strains showed production of trehalose glycolipids (from *Rhodococcus* sp.) and lipopeptide (from *Bacillus* sp.).

In sum, using oils and oil wastes to produce biosurfactants seems to be an interesting valorisation alternative, and also a good strategy of waste management for some industrial fields.

Biosurfactant production of agro-industrial wastes

Several agro-industrial wastes are also potential alternative raw materials for the production of biosurfactants. Potato process effluents (wastes from potato processing industries) were used to produce biosurfactant by *B. subtilis* [23, 128-131]. Potatoes are composed of 80% water, 17% carbohydrates, 2% protein, 0.1% fat and 0.9% vitamins, inorganic minerals and trace elements. Other carbohydrate-rich residues, such as cassava wastewater, have also been used for the production of surfactin by *B. subtilis* [132-134]. Another interesting alternative is the use of molasses, as this by-product of the sugar cane industry has many applications due to its low price as compared to other sources of sugar and the presence of several other compounds besides sucrose. These include minerals, organic compounds and vitamins, which are valuable for the fermentation process [20, 21, 31, 33, 148, 149]. The authors [20] studied different combinations of supplemented molasses medium as alternative substrates for biosurfactant production by *Lactococcus lactis* 53 and

Streptococcus thermophilus A. The biosurfactant production yields achieved with supplemented molasses medium were higher than the obtained whether with conventional or supplemented cheese whey medium. Although higher amounts of biosurfactant were produced with a medium composed of molasses (20 g/L sucrose) supplemented with 2.3 g/L yeast extract and 18 g/L peptone and with a medium composed of molasses (20 g/L sucrose) supplemented with 8.8 g/L yeast extract, 17.5 g/L peptone and 92.6 g/L sodium glycerophosphate for *L. lactis* 53 and *S. thermophilus* A, respectively; a better compromise between good yields and low costs is achievable with a medium where peptone and yeast extract amounts are lower (molasses (20 g/L sucrose) supplemented with 3 g/L yeast extract and 5 g/L peptone). Thus, an increase about 1.2 to 1.4 times in the mass of produced biosurfactant per gram cell dry weight and a 80% medium preparation costs reduction comparing with the synthetic MRS or M17 medium were achieved, for both strains. Solaiman and collaborators [150] used soy molasses and oleic acid as co-substrates for the production of sophorolipids by *C. bombicola*. Additionally, some authors refer to the utilization of lignocellulosic residues for biosurfactant production [34, 35]. Most agricultural wastes are made up mainly of cellulose, hemicelluloses, and lignin, and before fermentation, they have to be fractionated upon chemical and/or enzymatic stages to obtain sugar solutions, which (after nutrient supplementation) can be used as fermentative media for the production of biosurfactants. In their work, Moldes and co-workers tested barley bran, trimming wine shoots, corn cobs, distilled grape marc and *Eucalyptus globulus* chips and found that all of them except barley bran allowed interesting biosurfactant production yields [34, 35]. Comparative studies on the kinetic parameters of rhamnolipid production by *P. aeruginosa* using distillery and whey wastes as substrates were also reported [126, 151]. The results indicated that the kinetic parameters (specific growth rates and specific product formation rates) from both types of waste were comparatively better than the synthetic medium, revealing that both these industrial wastes (distillery and whey) can be successfully utilized as substrates for biosurfactant production.

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. Several other starchy waste substrates, such as rice water (effluent from rice processing industry and domestic cooking), cornsteep liquor, and wastewater from the processing of cereals, pulses and molasses, have tremendous potential to support microbial growth and biosurfactant production.

Biosurfactant production from cheese whey

A good substrate for biosurfactant production is whey, as it is composed of high levels of lactose, protein, organic acids and vitamins [15, 20, 78, 126, 127, 152]. Whey is a waste product from cheese production that represents a

major pollution problem for countries depending on dairy economics and is normally used as animal feed. To use the lactose effectively, a chosen organism must be able to consume the lactose and its breakdown products, glucose and galactose. Koch and co-workers [153] have developed a strain of *P. aeruginosa* to use whey for the production of rhamnolipids [153] and *B. subtilis* is also known to produce surfactin using this substrate [33, 40, 140].

In a study on the optimization of the media composition for the production of biosurfactants by lactic acid bacteria (*L. lactis* 53 and *S. thermophilus* A), Rodrigues and co-workers [152] achieved an increase about 2 times in the mass of produced cell-bound biosurfactant (milligram) per gram cell dry weight. Figure 1 and 2 shows the fermentation evolution for *L. lactis* 53 and *S. thermophilus* A respectively, as the result of the experimental design optimization of the media composition. It is not surprising the increase in the cell-bound biosurfactant mass recovery with the optimization procedure, as it is a growth-associated biosurfactant production and the cell growth was improved. However, it was interesting to notice that the change in the carbon source (from glucose to lactose) induced the cells to produce more biosurfactant. Lactic acid bacteria ferment sugars via different pathways and are also capable of forming other products, e.g. flavours such as diacetyl and acetoin, bacteriocins or biosurfactants. The different carbon sources give varying amounts of by-products [30]. Hence, it can be speculated that the use of lactose as carbon source instead of glucose induced the cells to use another metabolic pathway, and therefore the amount of mass of cell-bound biosurfactant produced milligram per gram cell dry weight varied. Based on these results, the authors [20] evaluated the use of cheese whey as an alternative media and compared with the conventional synthetic one. Several combinations of media supplementation were studied and, despite a higher biosurfactant production yield from *L. lactis* 53 was achieved with a medium composed of whey (50 g/L lactose) supplemented with 5.8 g/L yeast extract and 44.8 g/L peptone, an increase of 40% in the medium preparation costs comparing with the synthetic MRS medium was estimated due to the high amounts of peptone supplemented. Thus, a compromise situation must be established to obtain higher biosurfactant production yields with lower medium preparation costs. With another medium composed of whey (50 g/L lactose) supplemented with 3 g/L yeast extract and 5 g/L peptone, the mass of produced biosurfactant per gram cell dry weight increased 1.2 times with an estimated 60% decrease in the medium preparation costs comparing with the synthetic MRS medium. Similar conclusions were established for *S. thermophilus* A, where the use of a medium composed of whey (50 g/L lactose) supplemented with 3 g/L yeast extract and 5 g/L peptone, resulted in a biosurfactant production yield 1.5 times higher with an estimated 60% reduction in the medium preparation costs comparing with the synthetic M17 medium. In sum,

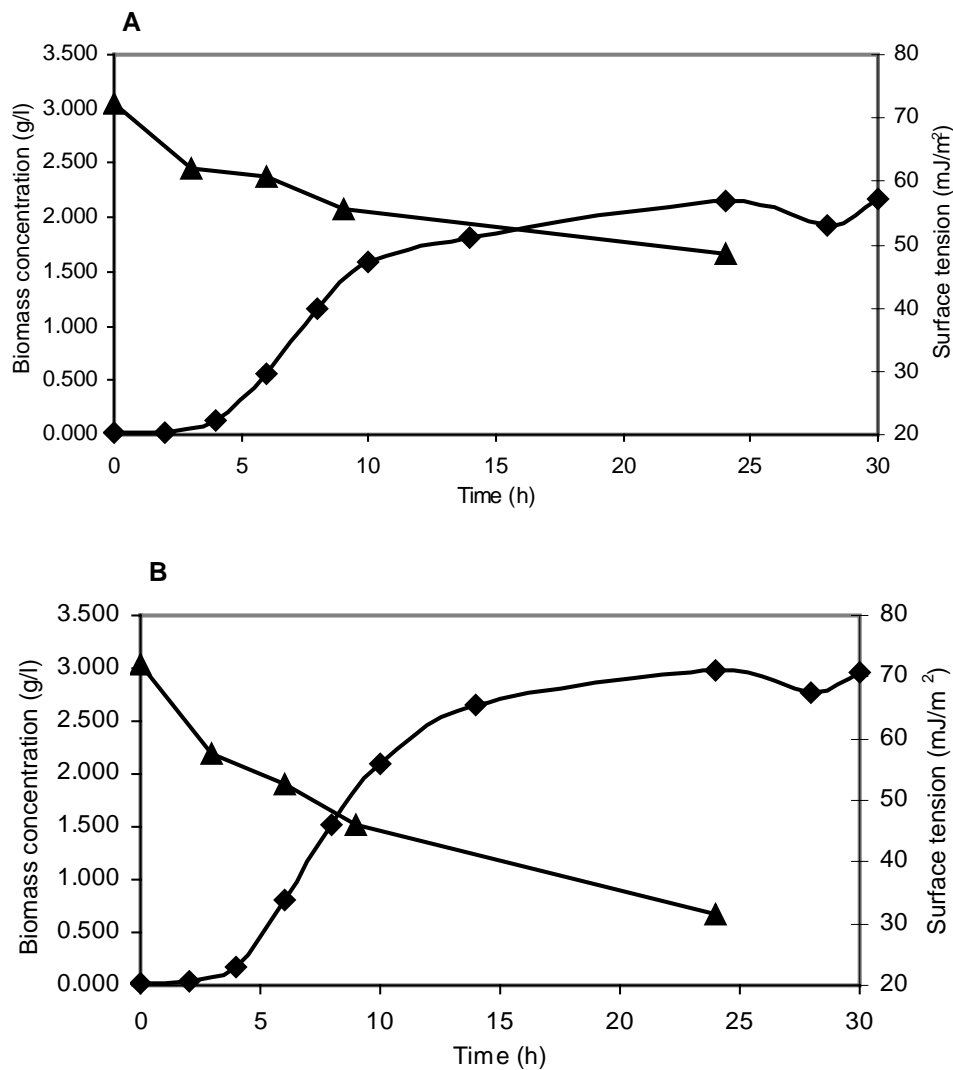


Figure 1. Fermentation evolution for *L. lactis* 53: variation of biomass concentration (g/l) (■) and surface tension (mJ/m²) (▲), in time. The biomass concentration is a measure of the cell growth, while surface tension is a measure of the biosurfactant activity. (Adapted from [152]) **A)** *L. lactis* 53 grown in MRS medium before experimental design optimization of the media composition. **B)** *L. lactis* 53 grown in MRS optimized by experimental design

the results achieved using the cheese whey as a substrate showed that the fermentations were carried out effectively with high yields and productivities of biosurfactant. Furthermore, other *Lactobacillus* strains have been screened for their ability to produce biosurfactants using lactose as carbon source [78]. The results achieved showed that *Lactobacillus pentosus* CECT-4023 is a strong biosurfactant producer strain. Therefore, the biosurfactant production from this strain using cheese whey as substrate was studied. Modelling of the biosurfactant production showed estimated values of maximum concentration of 1.4 g of biosurfactant/L and productivity of 0.093 g/(L.h).

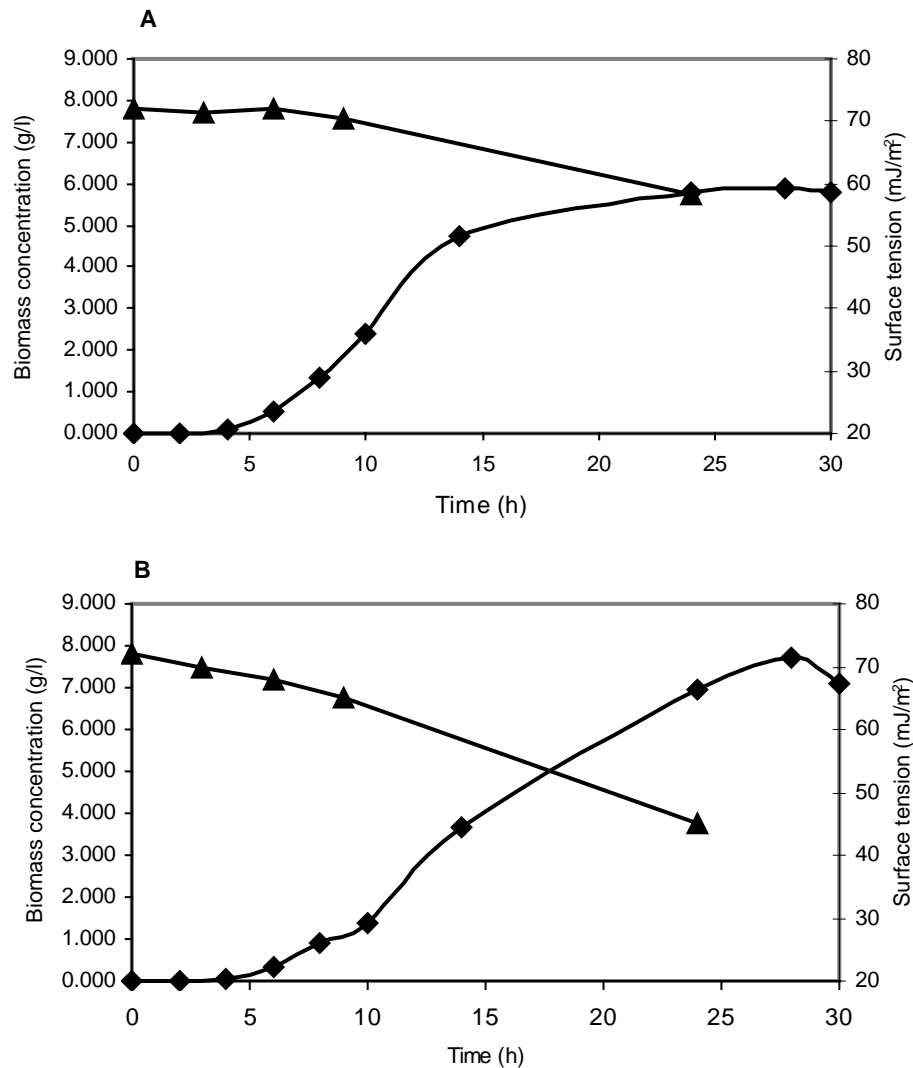


Figure 2. Fermentation evolution for *S. thermophilus* A: variation of biomass concentration (g/l) (■) and surface tension (mJ/m²) (▲), in time. The biomass concentration is a measure of the cell growth, while surface tension is a measure of the biosurfactant activity. (Adapted from [152]). **A)** *S. thermophilus* A grown in M17 medium before experimental design optimization of media composition. **B)** *S. thermophilus* A grown in M17 optimized by experimental design.

In addition, Daniel et al. [22, 136] achieved production of high concentrations of sophorolipids using a two-stage cultivation process: first, deproteinized whey concentrate (DWC) containing 110 g lactose was used for cultivation of the yeast *Cryptococcus curvatus* ATCC 20509; cells were then disrupted by passing the cell suspension directly through a high pressure laboratory homogeniser. After autoclaving, the resulting crude cell extract containing the single-cell oil served as a substrate for growth of *C. bombicola* ATCC 22214 and for sophorolipid production in a second stage. The

production and characterization of sophorolipids from whey was also reported by Otto and co-workers [24]. Crude sophorolipid mixtures showed moderate to good surface active properties (SFT^{min} 39 mN m⁻¹, CMC 130 mg l⁻¹), water solubilities (2–3 g l⁻¹) and low cytotoxicities (LC₅₀ 300–700 mg l⁻¹). In contrast, purified sophorolipids were more surface active (SFT^{min} 36 mN m⁻¹, CMC 10 mg l⁻¹), less water soluble (max. 70 mg l⁻¹) and showed stronger cytotoxic effects (LC₅₀ 15 mg l⁻¹). Incubation of crude sophorolipid mixtures with different hydrolases demonstrated that treatment with commercially available lipases such as from *Candida rugosa* and *Mucor miehei* distinctly reduced the surface active properties of the sophorolipids, while treatment with porcine liver esterase and glycosidases had no effect [24].

Conclusions

A host of interesting features of biosurfactants have led to a wide range of potential applications in the oil recovery, environmental bioremediation, food processing and medicine fields. These molecules comprise a range of remarkable characteristics such as high biodegradability, low toxicity, and effectiveness at extremes of temperature, pH and salinity. Although the biosurfactants reveal a high potential of application, the successful commercialization of every biotechnological product depends largely on its bioprocess economics. At present, the prices of biosurfactants are not competitive as compared with their chemical counterparts due to their high production costs and low yields. Therefore, major efforts have been reported in order to reduce their production and recovery costs. Strategies such as the use of cheaper substrates and optimization of their production coupled with novel and efficient multistep downstream processing methods, and the use of recombinant and mutant hyperproducing microbial strains, can make biosurfactant production economically feasible. Therefore, a variety of cheap raw materials, including plant-derived oils, oil wastes, starchy substances, cheese whey and distillery wastes have been reported to support biosurfactant production. Future biosurfactant research should, then, be more focused on the economics of biosurfactant production processes, particularly through the use of alternative low-cost fermentative media.

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