Chapter

Biosurfactants from Yeasts:
Characteristics, Production and Application

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Abstract

Biosurfactants are surface-active compounds from biological sources, usually extracellular, produced by bacteria, yeast or fungi. Research on biological surfactant production has grown significantly due to the advantages they present over synthetic compounds such as biodegradability, low toxicity, diversity of applications and functionality under extreme conditions. Although the majority of microbial surfactants have been reported in bacteria, the pathogenic nature of some producers restricts the wide application of these compounds.

A growing number of aspects related to the production of biosurfactants from yeasts have been the topic of research during the last decade. Given the industrial importance of yeasts and their potential to biosurfactant production, the goal of this chapter is to review the biosurfactants identified up to present, focusing the relevant parameters that influence biosurfactant production by yeasts and its characteristics, revealing the potential of application of such compounds in the industrial field and presenting some directions for the future development of this area, taking into account the production costs.

Introduction

Surfactants are amphiphilic compounds possessing both hydrophilic and hydrophobic moieties. They can reduce surface and interfacial tensions by accumulating at the interface between two immiscible fluids, thus stabilizing emulsions, or increasing the solubility of hydrophobic or insoluble organic compounds in aqueous media. They can be of synthetic or biological origin and the market for these compounds is on expansion.[1]

Due to their interesting properties such as lower toxicity, higher biodegradability, higher foaming capacity and higher activity at extreme temperatures, pH levels and salinity,[2] biosurfactants have been increasingly attracting the attention of the scientific community as promising candidates for the replacement of a number of synthetic surfactants. These compounds are biological molecules with noticeable surfactant properties similar to the well-known synthetic surfactants and they also include microbial compounds with surfactant properties.[3-6]

The majority of microbial biosurfactants described in literature is of bacterial origin and the genders most reported as biosurfactant producers are *Pseudomonas sp., Acinetobacter sp., Bacillus sp.* and *Arthrobacter sp*. However, due to the pathogenic nature of the producing organisms, the application of these compounds is restricted, not being suitable for use in food industry, among others.[7]

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The study of biosurfactant production by yeasts has been growing in importance, with production being reported mainly by the genders *Candida sp., Pseudozyma sp.* and *Yarrowia sp.* The great advantage of using yeasts in biosurfactant production is the GRAS (generally regarded as safe) status that most of theses species present, for example *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Organisms with GRAS status are not toxic or pathogenic, allowing the application of their products in the food and pharmaceutical industries.[8]

Biosurfactant Classification and Characteristics

Many microorganisms have the ability to produce molecules with surface activity. Two main types of surface-active compounds are produced by microorganisms: biosurfactants and bioemulsifiers.[9] Biosurfactants significantly reduce the air-water surface tension while bioemulsifiers do not reduce as much the surface tension but stabilize oil-in-water emulsions. During the last decades, there has been a growing interest in isolating microorganisms that produce surface active molecules with good surfactant characteristics such as low CMC and high emulsification activity, simultaneously presenting low toxicity and good biodegradability.[10]

Biosurfactants are categorized mainly by their microbial origin and chemical composition. Most extracellular yeast surfactants characterized and reported in literature have been identified as glycolipids, protein-carbohydrate-lipid or protein-carbohydrate complexes, lipids or fatty acids. Table 1 presents the yeast producing species identified up to present and the type of biosurfactant produced.

Glycolipidis biosurfactants are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. Among these the most interesting are the sophorolipids, which have been identified in *Torulopsis bombicola*,[11,12] *T. petrophilum*[13] and *T. apicola*[14,15] and consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxy fatty acid. Although sophorolipids can lower surface and interfacial tension, they are not very effective emulsifying agents.[16] Both lactonic and acidic sophorolipids were reported to lower the interfacial tension between n-hexadecane and water from 40 to 5 mN/m and to display remarkable stability toward pH and temperature changes.[13] Another gycolipid produced by yeasts, mannosylerythritol lipids, was identified from *Candida antarctica*[22] and *Pseudozyma rugulosa*[18] and exhibit excellent surface-active and vesicle forming properties.

Sarrubo et al[19] report the production of a biosurfactant from *Y. lipolytica* in the presence of glucose as carbon source, composed by 47% protein, 45% carbohydrate and 5% lipids. Although produced using the same carbon source but from a different *Y. lipolytica* strain, Yansan, a biosurfactant consisting of a polysaccharide-protein complex with negligible lipid content, shows a much lower protein content (15%).[9] Another *Y. lipolytica* derived surfactant,[20] produced with hexadecane as carbon source, was found to be also a lipid-carbohydrate-protein complex with an even lower protein content, 5% and very high lipid concentration, 75%. Liposan, a biosurfactant produced in the presence of a water-immiscible substrate by yet another *Y. lipolytica* strain, contained no lipid in its constitution, only carbohydrate (83%) and protein (17%) and the yeast seems not to be able to produce surfactants using glucose as carbon source.[21]

In what concerns their ability to produce and stabilize emulsions Liposan only displays emulsification activity with long chain hydrocarbons[3] while the surfactant produced by *Y. lipolytica* reported by Zinjarde et al[20] was not able to emulsify *n*-alkanes. Yansan was observed to present high emulsification activity with several hydrocarbons tested, including both aliphatics and aromatics.[6]

It has been demonstrated that the protein content of these polymers plays an important role in the emulsification activity. In fact, many mannoproteins extracted from yeasts' wall, have been reported to have high emulsification properties due to the presence of hydrophilic mannose polymers covalently attached to the protein backbone providing the amphiphilic structure common to surface-active agents.[22]

Table 1. Main biosurfactant producing yeast species

Biosurfactant	Producing Microorganisms	References
Sophorolipids	*Candida bombicola*	11
	Candida bombicola	12
	Torulopis petrophilum	13
	Candida (torulopsis) apicola	14
	Torulopsis apicola	15
	Candida bogorienses	65
Mannosylerythritol lipids	*Candida antarctica*	17
	Pseudozyma rugulosa	18
	Candida sp. SY16	44
	Pseudozyma aphidis	55
	Kurtzmanomyces sp. I-11	66
	Pseudozyma fusifornata, P. parantarctica, P. tsukubabaensis	67
Carbohydrate—protein—lipid complex	*Candida lipolytica UCP0988*	5
	Candida lipolytica IA 1055	19
	Yarrowia lipolytica NCIM 3589	20
	Debaryomyces polymorphus	68
	Candida tropicalis	68
Carbohydrate—protein –complex	*Candida lipolytica ATCC 8662*	3,21
	Yarrowia lipolytica IMUFRJ 50682	6
Mannanoprotein	*Saccharomyces cerevisiae*	22
	Kluyveromyces marxianus	27
ND[a]	*Candida utilis*	7
Fatty acids	*Candida ingens*	47
Lipids	*Rhodotorula glutinis*	69

[a]ND not determined.

Production Processes

The Influence of the Culture Medium Composition

Biosurfactants are produced by a number of yeasts, either extracellularly or attached to parts of the cell, predominantly during their growth on water-immiscible substrates. However, some yeasts may produce biosurfactants in the presence of different types of substrates, such as carbohydrates. The use of different carbon sources changes the structure of the biosurfactant produced and, consequently, its properties. These changes may be welcomed when some properties are sought for a particular application.[23] There are a number of studies in biosurfactant production involving the optimization of their physicochemical properties.[6,24,25]

The composition and characteristics of biosurfactants are also reported to be influenced by the nature of the nitrogen source as well as the presence of iron, magnesium, manganese, phosphorus and sulphur. The influence of the culture media on the biosurfactant production is discussed in detail below.

Carbon Source

Several reports in the literature address the influence of the carbon source in biosurfactant production by different yeast strains showing the possibility to use a wide variety of substrates.

Pareilleux[26] isolated surface-active compounds in the growth medium of *Candida lipolytica* using *n*-alkane as carbon source, but when this yeast was cultivated with glucose as carbon source no bioemulsifier was produced. In a similar study, Zinjarde and Pant[4] demonstrated that the surfactant biosynthesis by *Y. lipolytica* NCIM 3589 using soluble substrates such as glucose, glycerol, sodium acetate or alcohol was not viable. The authors identified, however, the presence of a bioemulsifier in culture media containing crude oil and alkanes (C_{10}-C_{18}).

Cirigliano and Carman[3] have also shown that a strain of *Y. lipolytica* produces biosurfactants through different carbon sources such as hexadecane, paraffin, soybean oil, olive oil, corn oil and cottonseed oil, with hexadecane identified as the best one.

Many vegetable oils (corn, soybean, sunflower and safflower) have been used as substrate for biosurfactant production by *T. bombicola*. The biosurfactants yield were similar for all the oils investigated.[16]

In 2001, Sarubbo et al[19] identified for the first time a biosurfactant produced by *Y. lipolytica* IA 1055 using glucose as carbon source. These authors demonstrated that the induction of biosurfactant production is not dependent on the presence of hydrocarbons. Another strain, *Y. lipolytica* IMUFRJ 50682, producing a bioemulsifier from glucose with high emulsification activity for oil-in-water emulsions was identified by our group.[6]

Lactose has also been used as soluble substrate for the production of compounds with emulsifying activity, such as the production of mannan-proteins by *Kluyveromyces marxianus*.[27]

Although it is also possible to produce biosurfactants in the presence of water soluble carbon sources, several studies show that often higher production yields are obtained when hydrophobic substrates are added.[3,4,26] A number of works describe the importance of combining a water insoluble substrate with a carbohydrate in the culture medium.

Biosurfactant production was identified on a *Candida glabrata* strain isolated from mangrove sediments. The maximum bioemulsifier production was observed when the strain was cultivated on cotton seed oil (7.5%) and glucose (5.0%), reaching values of 10 g l^{-1} after 144 hours. The cell-free culture broth containing the biosurfactant produced presented a surface tension of 31 mN m^{-1}.[25]

Casas and Ochoa[11] studied the medium composition of sophorolipids production by *Candida bombicola*. The carbon sources promoting the best biosurfactant production were glucose (100 g l^{-1}) and sunflower oil (100 g l^{-1}), used simultaneously, resulting in a biosurfactant concentration of 120 g l^{-1} after 144 hours of fermentation.

Sarrubo et al[5] investigated the production of a biosurfactant by *C. lipolytica* in a medium containing canola oil (100 g l^{-1}) and glucose (100 g l^{-1}). The surface-active compound produced was constituted by a protein—lipid—polysaccharide complex and was able to reduce the surface tension of water from 71 mN m^{-1} to 30 mN m^{-1}.

The hydrophilic substrates are initially metabolised by the microorganism for its energetic requirements and afterwards it also uses such substrates in the synthesis of the polar portion of the biosurfactant molecule. On the other hand, hydrophobic substrates are exclusively used for the production of the apolar moiety of the biosurfactant. The *Candida* species seem to be capable to incorporate fatty acids directly into the production of biosurfactants.[15]

There are various pathways for the synthesis of the two main parts that constitute a biosurfactant molecule and they are generally accomplished through specific sets of enzymes. In most cases, the first enzymes used in the synthesis of these precursors are regulatory ones; therefore, in spite of their diversity, there are some common features to the synthesis of biosurfactants and

regulation.[28] According to Syldatk and Wagner,[29] the synthesis of the different moieties of biosurfactants and their linkage follows one of four possible paths: (i) the hydrophilic and hydrophobic moieties are synthesized de novo by two independent pathways; (ii) the hydrophilic moiety is synthesized de novo while the synthesis of the hydrophobic moiety is induced by substrate; (iii) the hydrophobic moiety is synthesized de novo, while the synthesis of the hydrophilic moiety is substrate dependent; and (iv) the synthesis of both the hydrophobic and hydrophilic moieties are substrate dependent.

One important factor affecting the surfactant synthesis is naturally the alkyl chain length of the compounds used as carbon source. Kitamoto et al[30] studied the production of mannosylerythritol lipids (MEL), a biosurfactant produced by *C. antarctica*, using different *n*-alkanes as carbon source. The authors found that *C. antarctica* T-34 did not grow well on *n*-alkanes (3%, v/v) ranging from C_{10} to C_{18} and no MEL were produced. However, it was possible to successfully produce MEL from *n*-alkanes ranging from C_{12} to C_{18} using 13.6 g resting cells l^{-1}. Only small quantities of MEL were obtained from *n*-alkanes longer than nonadecane (C_{19}), probably due to their high melting points, above the culture temperature of 30°C. The authors observed that the productivity of MEL was markedly affected by the chain-length of the alkane substrates, with the highest productivity obtained from *n*-octadecane. Cavalero and Cooper[12] have shown that the sophorolipid yield from *C. bombicola* ATCC 22214 increases with the *n*-alkane chain length (from C_{12} to C_{15}). However, Zinjard and Pant[4] demonstrated that the production of a biosurfactant by *Y. lipolytica* did not change when using different *n*-alkanes as substrate.

Carbon Source from Renewable Resources

To date, biosurfactants are unable to compete economically with chemically synthesized compounds available in the market, due to their high production costs resulting from the use of expensive substrates. These costs may be significantly reduced by the use of alternative sources of nutrients with lower costs and by reaching high yields of product.[31] A possible solution for the first approach would be the re-utilization of industrial wastes, for instance, the agro-industrial or the oil-containing wastes. This strategy decreases the costs of biosurfactant production, reducing simultaneously, the pollution caused by the waste disposal in landfills.[32]

Many food industries using fats and oils, generate large quantities of wastes, tallow, lard, marine oils or soapstock and free fatty acids from the extraction of seed oils. Waste disposal is a growing problem, which explains the increasing interest in the waste valorisation through microbial transformation.[33]

Oil refinery waste, either with soapstock or postrefinery fatty acids, was used by Bednarski et al[34] to synthesize surfactants in the cultivation medium of *C. antarctica* or *Candida apicola*. The authors showed that the production of glycolipids, in a medium supplemented with soapstock and postrefinery fatty acids, was 7.5 to 8.5-fold greater then in the medium without addition of oil refinery waste.

The soy molasses, a by-product from the production of soybean oil, plus oleic acid were tested as carbon sources for the production of sophorolipids by the yeast *C. bombicola*.[35] The authors reported a production of 21 g l^{-1} after seven days of fermentation, which is a low value when compared to the production in the presence of glucose and oleic acid (79 g l^{-1}).

Used frying oil is produced in large quantities both in the food industry and from domestic uses. Haba et al[36] compared the composition of used olive and sunflower oils with the standard unused oils in their study and found that the most important difference is the presence of 22.52 wt% of fatty acids of low chain length ($<C_{14}$) in used oil. Thanomsub et al[37] isolated yeast strains from plant material in Thailand and a strain of *Candida ishiwadae* was able to produce glycolipid biosurfactants from used soybean cooking oil. The biosurfactants produced were characterized to be monoacylglycerols and exhibited high surfactant activities.

The production of sophorolipids by *C. bombicola* was stimulated by the addition of animal fat, a residue of the meat processing industry. A high biosurfactant production (120 g l^{-1}) was obtained after 68 hours of fermentation.[38]

Nowadays, a very important renewable origin carbon source is glycerol. The increase in the world production of biodiesel is generating large quantities of raw glycerol, which is a by-product from this bio-fuel production. With the production of 10 kg of bio-diesel from rapeseed oil, 1 kg of glycerol becomes available[39] and its price is decreasing and tends to decrease even more as the traditional glycerol markets become saturated. Morita et al[40] used glycerol for the production of glycolipids by *Pseudozyma antarctica*, obtaining 16.3 g l^{-1} of biosurfactant after seven days of fermentation. The biosynthesis of sophorolipids by *C. bombicola* was also studied in the presence of a by-product of bio-diesel production, with 40% of glycerol and 34% of hexadecane soluble compounds (92% of fatty acids and 6% of monoacylglycerol/triacylglycerol) and 26% of water. The fermentation yielded 60 g l^{-1} of sophorolipids.[41]

Another good substrate for biosurfactant production is lactic whey. It is composed of high levels of lactose (75% of dry matter), 12-14% protein, organic acids and vitamins. Disposal of whey is a major environmental problem for countries depending on dairy economics.[33] Daniel et al[42] achieved production of high concentrations of sophorolipids (422 g l^{-1}) using a two-stage cultivation process: first, deproteinized whey concentrate (DWC) containing 110 g lactose was used for cultivation of *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.

Nitrogen Source

Nitrogen is important in the biosurfactant production medium because it is essential for microbial growth as protein and enzyme syntheses depend on it. Different nitrogen compounds have been used for the production of biosurfactants, such as urea,[43] peptone,[44] yeast extract,[11,45-47] ammonium sulphate,[20] ammonium nitrate,[37] sodium nitrate,[34] meat extract and malt extract,[48] etc. Yeast extract is the most used nitrogen source for biosurfactant production, but its concentration depends on the microorganism and the culture medium.

Cooper and Paddok[16] have studied the effect of the nitrogen source, using sodium nitrate, ammonium chloride, ammonium nitrate, urea or yeast extract in the biosurfactant production by *T. bombicola* in agitated flasks. The authors observed that nitrate was not a good nitrogen source since it affected the biomass growth while 5 g l^{-1} of yeast extract promoted a higher surfactant production. When the yeast extract was substituted by peptone, the biosurfactant concentration obtained was reduced to half and a very low concentration was obtained when urea was used.

The production of a bioemulsifier by *Y. lipolytica* was also evaluated using different nitrogen sources: ammonium sulphate, ammonium chloride, ammonium nitrate, urea and sodium nitrate. The results showed that ammonium sulphate and ammonium chloride were the best nitrogen sources for the emulsifier production. The emulsifying activity was reduced to half when ammonium nitrate and urea were used and no emulsifying activity was detected in the culture medium with sodium nitrate.[20]

Casas and Ochoa[11] tested different yeast extract concentrations (1 to 20 g l^{-1}) to optimize the formulation of the culture medium of *C. bombicola* and described that the production of sophorolipids is better in the presence of low yeast extract concentration (1 g l^{-1}). According to the authors when high yeast extract concentrations are used, the biosurfactant production decreases because the carbon source is used in yeast growth.

Johnson[49] reported the influence of the nitrogen source in the production of a biosurfactant by the yeast *Rhodotorula glutinis* IIP-30. The author revealed that the use of potassium nitrate presented the best result in comparison to other nitrogen sources (ammonium sulphate and urea).

As shown in literature, several researchers choose to use more than one nitrogen source, obtaining good surfactant concentrations. Lukondeh et al[27] investigated the production of a biosurfactant by *K. marxianus* FII 510700, by using yeast extract (2 g l^{-1}) and ammonium sulphate (5 g l^{-1}) as nitrogen sources. The bioemulsifier produced presented high emulsification activity (around 76% of emulsion phase after 90 days at 4°C).

To optimize the production of a bioemulsifier by *C. lipolytica*, Alburquerque et al[50] used a factorial experimental design to investigate the effect and the interaction between urea, ammonium sulphate, potassium dihydrogen orthophosphate and corn oil in the emulsifying activity of the culture medium. Ammonium sulphate, potassium dihydrogen orthophosphate and corn oil had a positive effect in emulsifying activity while urea presented a negative effect.

The production of surface-active compounds often occurs when the nitrogen source is depleted in the culture medium, during the stationary phase of cell growth.[44] Kitamoto et al[22] studied the cell growth of *C. antarctica* and its biosurfactant production in a culture medium containing the ammonium ion (10 g l^{-1}) and peptone (1 g l^{-1}) as nitrogen sources. The authors noticed that the production of glycolipids starts when the nitrogen source is exhausted after 50 hours of fermentation, reaching a concentration value of 38 g l^{-1} after 200 hours of fermentation.

In the same line of thought, Albrecht et al[51] suggested a mechanism in which the biosurfactant synthesis happens in limiting nitrogen conditions. According to the authors, this condition causes the decline of the specific activities of NAD- and NADP-dependent isocitrate dehydrogenase, which catalyses the oxidation of isocitrate to 2-oxoglutarate in the citric acid cycle. With the reduction of the activity of this enzyme, isocitrate accumulates, leading to the accumulation of citrate in mitochondria. Citrate and isocitrate are, then, transported to the cytosol, where the first it is cleaved by citrate synthase, forming acetyl-CoA, which is the precursor of fatty acids synthesis and, therefore, biosurfactant production increases.

An important parameter studied by several researchers is the quantitative ratio between carbon and nitrogen sources (C/N) used in biosurfactant production. Different C/N ratios and hydrocarbons were used in biosurfactant production by *C. tropicalis*. The emulsifying activity rose with the increase in C/N ratio for almost all cases, when nitrogen was the limiting factor.[52] Similar results were obtained by Jonhson et al[49] for the production of a biosurfactant from *R. glutinis*.

Table 2 presents a compilation of literature data on biosurfactant production by yeasts, including parameters such as the carbon and nitrogen sources concentration, the yield of the production (Yp/s) and volumetric productivity (Qp).

The Environmental Factors Affecting the Production

Environmental factors are extremely important in the yield and characteristics of the biosurfactant produced.[53] In order to obtain large quantities of biosurfactant it is necessary to optimize the process conditions because the production of a biosurfactant may be induced by changes in pH, temperature, aeration or agitation speed.

pH

The effect of pH in the biosurfactant production by *C. antarctica* was 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.[30]

Zinjarde and Pant[4] studied the influence of initial pH in the production of a biosurfactant by *Y. lipolytica*. The authors observed that the best production occurred 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 glicolipids by *C. antarctica* and *C. apicola*. When pH was maintained at 5.5, the production of glicolipids reached a maximum. Without the pH control, the synthesis of the biosurfactant decreased.[34]

The production of a bioemulsifier by *R. glutinis* during feed batch fermentation was significantly influenced by both pH and temperature, with the optimum conditions at 30˚C and pH 4.0.[49]

Temperature

Most biosurfactant productions reported were performed in a temperature range of 25 to 30˚C. There are many works in literature reporting the influence of this parameter. Casas and Ochoa[11] showed that the amount of sophorolipids obtained in the culture medium of *C. bombicola* at temperature of 25˚C or 30˚C was similar. Nevertheles, the fermentation performed at 25˚C presented a lower biomass growth and a higher glucose consumption rate in comparison to the

Table 2. Data from biosurfactant production by yeasts reported in literature

Strain	Carbon Source ($g\ l^{-1}$)	Nitrogen Source ($g\ l^{-1}$)	PB[a]	T[b] (h)	Operation Mode	$Y_{P/S}$[c] ($g\ g^{-1}$)	X[d] ($g\ l^{-1}$)	$Y_{P/X}$[e] ($g\ g^{-1}$)	Qp ($g\ (l\ h)^{-1}$)	Reference
Torulopsis bombicola ATCC22214	Glucose (500) Safflower Oil (1000)	Yeast extract (5)	18 $g\ l^{-1}$	48	Batch	0.012	12.4	1.45	0.375	16
Candida bombicola ATCC22214	Glucose (100) Animal fat (100)	CSL[f] (4) Urea (1.5)	120 $g\ l^{-1}$	68	Batch	0.6	30	4	1.76	38
Candida tropicalis ATCC20336	n-hexadecane (10)	Yeast extract (0.3) Peptona (0.5)	0.81 U	72	Batch	NDg	ND	ND	ND	68
Debaryomyces polymorphus ATCC20499	n-hexadecane (10)	Yeast extract (0.3) Peptone (0.5)	0.11 U	72	Batch	ND	ND	ND	ND	68
Candida sp. SY 16	Glucose (15) Soybean oil (15)	Peptone (1)	95 $g\ l^{-1}$	200	Feed-batch	0.475	15.0	6.33	0.475	44
Candida bombicola NRRL Y-17069	Glucose (100) Sunflower oil (100)	Yeast extract (1)	120 $g\ l^{-1}$	192	Batch—Resting cell	0.6	23.0	5.21	0.625	11
Candida antarctica ATCC20509	Soybean oil (80)	Yeast extract (1)	46 $g\ l^{-1}$	144	Batch	0.57	28.4	1.61	0.32	45
Candida antarctica ATCC20509	Soapstock (100)	Yeast extract (1) $NaNO_3$ (2)	15.9 $g\ l^{-1}$	85	Feed-batch	0.636	1.8	8.83	0.1870	34
Candida antarctica T-34	Nut oil (80)	Yeast extract (1)	47 $g\ l^{-1}$	144	Batch—Resting cell	0.587	24	1.95	0.326	17

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Table 2. Continued

Strain	Carbon Source ($g\ l^{-1}$)	Nitrogen Source ($g\ l^{-1}$)	PB[a]	T[b] (h)	Operation Mode	$Y_{P/S}$[c] ($g\ g^{-1}$)	X[d] ($g\ l^{-1}$)	$Y_{P/X}$[e] ($g\ g^{-1}$)	Qp ($g\ (l\ h)^{-1}$)	Reference
Candida antarctica T-34	n-octadecane (60)	Yeast extract (1) $NaNO_3$ (2)	40.5 g l^{-1}	144	Batch—Resting cell	0,675	16	2.53	0.281	30
Candida ingens CB-216	Corn oil (20)	Yeast extract (2)	5.6 g l^{-1}	168	Batch	0,28	23.9	0.234	0.033	47
Candida lipolytica ATCC 8662	n-hexadecane (10)	Yeast extract (6)	1.3 U/mL	130	Batch	ND	ND	ND	ND	3
Yarrowia lipolytica NCIM3589	Hexadecano (10)	$(NH_4)_2SO_4$ (5)	3.0 U/mL	144	Batch	ND	3	ND	ND	4
Candida lipolytica IA 1055	Babassu oil (50)	Urea (0.25)	0.66 U ml^{-1}	60	Feed-batch	ND	6 UFC ml^{-1}	ND	ND	43
Yarrowia lipolytica IMUFRJ 50682	Glucose (20)	Peptone (6,4) Yeast extract (10)	2.0 U ml^{-1}	170	Batch	ND	ND	ND	ND	6
Candida lipolytica UCP0988	Glucose (100) Canola oil (100)	Yeast extract (2)	8 g l^{-1}	48	Batch	0,04	ND	ND	0.166	5
Kluyveromyces marxianus FII 510700	Lactose (40)	Yeast extract (2) $(NH_4)_2SO_4$ (5)	ND	ND	Batch	ND	ND	ND	ND	27
Candida apicola ATCC 20509	Soapstock (100)	Yeast extract (1) $NaNO_3$ (2)	10.3 g l^{-1}	144	Batch	0,103	6.5	9.70	0.071	34
Candida. ishiwadae	Soybean frying oil (40)	Yeast extract (0.5) NH_4NO_3 (3)	0.25 μg	168	Batch	ND	ND	ND	ND	37

[a]BP: biosurfactant production; [b]T: biosurfactant production time; [c]$Y_{P/S}$: biosurfactant yield related to substrate consumption; [d]X: cell concentration produced; [e]$Y_{P/X}$: biosurfactant yield related to cell produced; [f]CSL: corn steep liquor; [g]ND: not determined.

fermentation performed at 30˚C. In a similar study, Desphande and Daniels[38] observed that the growth of *C. bombicola* reached a maximum at a temperature of 30˚C while 27˚C was 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. When resting cells were used the production occurred in a large range of temperature. The effect of aeration was also investigated using different volumes of medium (20 to 60 ml) in flasks of 300 ml. The best yield was obtained with 30 ml of medium volume, which implies a medium—flask volume ratio of 0.1, demonstrating the importance of aeration in such systems.[22]

Aeration and Agitation

Aeration and agitation rates are important factors that influence the production of biosurfactants, since they facilitate the oxygen transfer from the gas phase to the aqueous phase and it may also be linked to the physiological function of microbial emulsifiers. It has been suggested that the production of bioemulsifiers can enhance the solubilization of water-insoluble substrates and, consequently, facilitate nutrient transport to microorganisms. Therefore, higher shear stress may induce surfactant secretion as the contact of organic droplets dispersed in water with microorganisms becomes more difficult. Desai and Banat,[28] in their review, reported some opposite results regarding *Norcadia erythropolis* and *Acinetobacter calcoaceticus* which produced less biosurfactant due to the increase of shear stress. They mention, however, that biosurfactant production with yeasts generally increases with stirring and aeration rates.

Adamczak and Bednarsk[45] evaluated the influence of aeration in the biosurfactant synthesis by *C. antarctica* and observed that the best production (45.5 $g\,l^{-1}$) was obtained when air flow rate was 1 vvm and the dissolved oxygen concentration was maintained at 50% of saturation. Nevertheless, changing the air flow rate to 2 vvm, there was a high foam formation and the biosurfactant production decreased 84%. The formation of foam is not appropriated for biosurfactant production because it removes biosurfactant, some biomass and lipids from the culture medium.

The effect of aeration rate was also investigated by Guilmanov et al[54] in agitated flasks. The best yield of sophorolipids produced by *C. bombicola* was reached with aeration rate between 50 and 80 mM of $O_2\,l^{-1}\,h^{-1}$.

Kinetics and Operation of Biosurfactant Production Process

It is very difficult to draw general guidelines for optimal biosurfactant production by yeasts because the biosurfactants identified are from a diverse group of compounds produced by a variety of microbial species. The process must, therefore, be optimized on a case by case basis. Most biosurfactants are excreted into the culture medium either during the exponential phase or at the stationary phase.[1] Cirigliano and Carman[3] showed that the emulsifier production by *C. lipolytica* IA 105530 was detected when the microorganism growth rate decreased but Amaral et al[6] described a growth-associated bioemulsifier production by *Y. lipolytica* IMUFRJ 50682 using a different carbon source.

T. bombicola produced most of the surfactant in the late exponential phase of growth. Therefore, Cooper and Paddock[16] proposed to grow the yeast on a single carbon source and then add a second type of substrate after the exponential growth phase, causing a burst of glycolipids production. The maximum yield was 70 $g\,l^{-1}$ or 35% of the weight of the substrate used. An economic analysis demonstrated that this biosurfactant could be produced at significantly lower cost than any of the previously reported microbial surfactants.

Biosurfactant production by resting or immobilized cells is a type of process in which there is no cell multiplication. Nevertheless, the cells continue to utilize the carbon source for their maintenance and for the synthesis of biosurfactants.[28] Several examples of biosurfactant production by resting cells are known. They include production of sophorolipids by *T. bombicola*[11] and *C. apicola*[14] and mannosylerythritol lipid production by *C. antarctica*.[17] Biosurfactant production by

resting cells is important in the reduction of costs associated with product recovery, as the growth and the product formation phases can be separated.

Besides the optimization of the culture medium, considerable attention has been directed towards the different ways to operate the biosurfactant production process. The production of biosurfactant by yeasts is generally carried out in batch or feed-batch fermentation, as can be noticed from Table 2. Batch and feed-batch fermentations are ways to operate bioprocesses which gather some advantages such as simpler equipments and less contamination problems. Some promising results with batch operations for biosurfactant production have been reported. For example, the production of mannosylerythritol lipid by *C. antarctica* achieved a concentration of 95 g l^{-1} after 200 hours of feed-batch fermentation using glucose and soybean oil as carbon source. The yield obtained by feed-batch fermentation was 2.6 times greater than in batch fermentation.[44] Rau et al[55] reported the production of a biosurfactant by *Pseudozyma aphidis* using soybean oil and glucose in a feed-batch fermentation, obtaining a high production (168 g l^{-1}) after eleven days of fermentation.

Other fermentation techniques include the application of air lift fermentor and continuous operation, which can improve biosurfactant production.[1] However these techniques haven't been used for biosurfactant production by yeasts. Therefore, the study of the optimization of the production operation is still lacking.

Potential Commercial Applications

Biosurfactants find applications in a wide variety of commercial areas and industrial processes such as oil recovery enhancement,[1] bio-remediation of oil-polluted soil and water,[56] replacement of chlorinated solvents used in the cleaning up of oil-contaminated pipes,[57] use in the detergent industry[58] and in the formulation of oil-in-water emulsions in the food, biotechnological, pharmaceutical and cosmetic industries.

Synthetic surfactants are commonly used in oils spills to disperse the oil and accelerate its mineralization, being the limiting steps determined by desorption and/or solubilization rates in soil and water. Surfactants increase the aqueous solubility of hydrophobic contaminants and, consequently, their microbial degradation.[2] The main problem associated to the use of synthetic surfactants is their toxicity, representing an additional source of environmental contamination. Thus, in the bioremediation field, biosurfactants come up as a major candidate for the replacement of synthetic surfactants due to their lower toxicity, high biodegrability and the possibility of in situ production.[1] The effects of biosurfactants on the biodegradation of petroleum compounds were investigated by Hua et al.[59] They reported that the addition of a biosurfactant produced by *Candida antarctica* T-34 could improve the biodegradation rate of some n-alkanes (90.2% for n-decane, 90.2% for n-undecane, 89.0% for dodecane), a mixture of n-alkanes (82.3%) and kerosene (72.5%) and showed that this biosurfactant could substitute with advantage synthetic surfactants.

The application of biodegradable and nontoxic biosurfactants can also be of substantial benefit in the biomedical and biotechnological fields. Perfluorocarbon-based emulsions are being exploited as oxygen injectable carriers, contrast agents, drug delivery systems or as cell culture media supplements and the employment of a nontoxic and biodegradable biosurfactant can lead to further improvements in these areas.[60]

Biosurfactants have also showed several promising applications in the food industry as food additives. Lecithin and its derivatives, fatty acid esters containing glycerol, sorbitan, or ethylene glycol and ethyoxylated derivatives of monoglycerides are currently in use as emulsifiers in the food industries worldwide. A bioemulsifier from *Candida utilis* has shown potential use in salad dressing[7] and good results were obtained with mannoproteins extracted from *S. cerevisiae* cell wall in mayonnaise formulation.[61]

Biosurfactants can also have a very important position in the cosmetic industries. A product containing 1 mol of sophorolipid and 12 mol of propylene glycol has excellent skin compatibility

and is used commercially as a skin moisturizer.[62] Sophorolipids are being produced and used by Kao Co. Ltd. as a humectant in cosmetics.[2]

Some other potential commercial applications of biosurfactants currently under study include the pulp and paper industry, textiles, ceramics and uranium ore processing.[28]

Conclusions

In the last few years, there has been an increase in the number of publications in the area of yeast biosurfactant production. This is a clear indication that these compounds are showing to be of interest in several areas and becoming technologically important. Despite the advantages of biosurfactant synthesis, its industrial use is still limited due to the costs involved in the production process.

The optimization of the production process is the key factor to improve yield and to reduce costs. Estimates show that the utilization of renewable source as substrates may reduce up to 30% of the production costs. Factors that influence biosurfactant production, such as nitrogen source, carbon source, pH, temperature, agitation and aeration, are also relevant on the optimization of the production and thus on the production cost.

The current cost of sophorolipids production by the yeast *C. bombicola* varies from 2 to 5 € kg^{-1}, depending on the substrate cost and the production scale,[63] while the market price of synthetic surfactants is around 2 € kg^{-1}. Some specific industrial sectors, such as cosmetic and pharmaceutical, biosurfactants have high application potential and will probably play a major role in short period of time, because of its enhanced characteristics and the high margins and value of the final product.

Nowadays, researches are slowly progressing to the genetic engineering through the use of recombinant DNA techniques for the manipulation of biosurfactant production.[64] These studies open new perspectives to increase production yields and might become the instrument to overcome the limitations for biosurfactants industrial application.

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