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Culture Media Formulation and Growth Conditions for Biosurfactants Production by Bacteria

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Abstract

At present, several industrial cities are showing a lot of pollution problems due to xenobiotics in soil. Most of them are recalcitrant to the environment dueto their low 1-octanol-water partitioning coefficient (hydrophobic organic contaminant),in addition they are toxic to the biota. Nowadays, the technique that is most compatible with the environment is bioremediation, which is used to remove these kinds of toxics from different matrixes. For this reason, a number of microorganisms where selected not only because they produce changes in the molecular structures of the toxics (through their enzymatic activity), but also because of the extra and intra cellular surface active compounds (biosurfactants)that they generate, containing tensoactive properties which are very similar to synthetic surfactants, that are involve in micelles the toxic from the soil, improve their desorption or their biodegradation by the same or by other microorganisms, and in this case the most studied where bacterial microorganisms. In order to induce the production of these substances in microorganisms, vast research had to previously be done; the aim of this work was to generate an overview of some of these important research projects, to be able to understand the growth conditions and nutritional requirements of the different microorganisms that produce biosurfactants as well as to outline the possibility of using them in the bioremediation process.

Keywords: Bacteria; Biosurfactants; Cell culture; Nutrients; Environmental conditions

Introduction

There are various microorganisms capable of synthesizing a wide spectrum of biosurfactants (bio-emulsifiers)-of either a high or a low molecular weight class. Low molecular weight biosurfactants are mostly glycolipids (trehalolipids, sophorolipids and rhamnolipids), however, they could also be lip peptides such as Surfactant, Gramicidin and Polymyxin. Heavier molecular biosurfactants include amphiphilicpolysaccharides, proteins, lip polysaccharides, lipoproteins or complex mixtures of these polymers [1]. The lighter ones reduce surface tension while the heavier ones, produce stable emulsions of water in oil [2]. Rhamnolipidsare made up of either one or two rhamnose molecules linked to one or two molecules of ß-hydroxypentanoic acid. Rhamnose with glycolipids was first described in *Pseudomonas aeruginosa* Trehalolipids, it is formed by trehalose disaccharides linked by mycolic acids onto C-6 and C-6'and it is associated with most species of *Mycobacterium, Nocardia* and *Corynebacterium;* threalose lipids from *Rhodococcuserythropolis* and Arthrobactersp. Lowered the surface and the interfacial tension. Sophorolipids contain dimeric sophorose carbohydrates linked to long chains of aliphatic (hydroxyaspartic) acids which are mainly produced by yeast (*Torulopsisbombicola*, *T. petrophilum* and *T. apicola*) [1]. Biosurfactants have better surface and interface activity than synthetic surfactants, these properties result in strong tolerance to high temperatures and salinity. Biosurfactants are biodegradable in soil or water, and their emulsion can be broken easily [3].

At present, bacteria are the focus of researchers investigating and working on the production of biosurfactants. Among those microorganisms that are being deeply studied we find *Pseudomonas aeruginosa* which consistently producer hamnolipids from various sources of carbon as shown by numerous studies. Its most negative aspect is the fact that it is inconvenient for use given its pathogenicity for humans. There are numerous studies about secondary metabolism biosynthetic pathways through which biosurfactants are produced. It is possible to establish that bacteria metabolism may shift toward sources of carbon in order to produce biosurfactants. In order to obtain energy and to produce biosurfactants from carbohydrates, the catalytic cycle of tricarboxylic acid (CTA) must occur. One of the enzymes from the CTA cycle is dehydrogenate is ocitrate which correlates to the production of biosurfactant.

It has been proven that this enzyme is of vital importance in the production of polihydroxibutirate (PHB) produced by the *Azotobacterbiejerinckii*.

To be able to redirect the metabolism of microorganisms towards the production of biosurfactants in a liquid culture, various nutritional and environmental conditions must be administered. Some of the most popular variables are the carbon source, nitrogen source, and environmental variables [4-12]. In the following paragraphs are presented some research with bacterial that produce biosurfactants.

Nutritional Factors

Carbon Sources: In order to produce biosurfactants from *Pseudomonas*spp the following water soluble carbon sources have been deployed: glycerol, glucose, mannitol and ethanol. Production levels of biosurfactants fall when using carbon sources which are immiscible in water such as olive oil and alkanes. Also, it has been shown that the various sources of carbon used by microorganisms modify the composition of biosurfactants in its polar fraction while keeping the glycolipid chain's length at the non-polar fraction unchanged (fatty acids). Nonetheless, a qualitative variation exists among biosurfactant production by *Acinetobacter*sp strains H13-A and H01-N as is evidenced by the number of alkane carbons [13]. *Bacillus subtilis* ATCC 21332 was used as a carbon source potato (60 g/L) for the production of Surfactin (decreased surface tension of the medium was 41.8 mN/m) [14]. Linhardt et al. [15], observed than *Pseudomonas aeruginosa* strain U129791, used corn oil as a carbon source and produced rhamnolipids, the highest production of rhamnose (5.4 g/L) was obtained when the concentration in culture medium was 40 g/L of corn oil. *Cellulomonascellulans* produced glycolipids (8.9 g/L, expressed as glucose) when it grew in liquid medium with 30 g glycerol/L [16].

P. aeruginosa 44T1 used hydrocarbons with carbon chains C12 and olive oil as carbon sources to produce rhanmolipids. However, in the presence of fructose, production is inhibited [17,18]. Benincasa et al. [19] proved that *P.aeruginosa* LB1 uses oapstock (3g/l) as carbon source to produce rhamnolipids (12 g/L). *Torulopsismagnolie* employslong-chain fatty acids, hydrocarbons and glycerin to produce So phorolipids [20]. When *Arthrobacterparaffineus* ATCC 19558 grows in D-glucose and when hexadecane is added during its latent growth phase, biosurfactant performance has a significant peak. Patel and Desai [21] reported that *Pseudomonas aeruginosa* GS3 increased their biosurfactant production with molasses (48%w/v of carbohydrates) present in the medium at 7% as a carbon source, which generated 0.24 g/L of rhamnose after 96h of incubation. Pruthi and Cameotra [22] reported that *Serratiamarcescens* produced biosurfactant (30 mN/m) using minimal medium containing sucrose (2% w/v) at a wide pH (2 - 12) and temperature (10-120°C) range.n-hexadecane (2% v/v) was used as the sole carbon source by *Arthrobacterprotophormiae* MTCC 688 to produce biosurfactant at different salt concentrations (10-100 g/L), where the highest concentration (5.3 g/L) was obtained using 20 g/L of salt (NaCl) at psychrophilic conditions (10°C) [23]. Chayabutra et al. [24] examined different carbon substrates (palmitic acid, stearic acid, oleic acid, linoleic acid, glycerol, vegetable oil, hexadecane and glucose) for *P. aeruginosa* in anaerobic and aerobic conditions. All of these carbon sources were able to support cell growth, and synthesis of rhamnolipids was only effective when using hexadecane, palmitic and stearic acid.

Hartoto and Mangumwidjaja (2002) studied the biosurfactant production (lipopeptides) ability of *Bacillus*sp BMN14 using different sugars (glucose, fructose, sucrose, etc.) at differ concentrations (1, 2, 3, 4, 5, y 6%, in the liquid medium) as carbon sources, and observed that the highest biomass (6.35 g/L) and biosurfactant production (2.23 g/L), the maximum specific growth (0.065/h), and the lowest surface tension (29 mN/m) were obtained with glucose at 4%. Yateem et al. [25] investigated the biosurfactant production by two different strains of *Pseudomonas aeruginosa* (KISR C1 and KISR B1) using crude oil, olive oil, hexadecane, diesel, kerosene and oil sludge, the results showed that the highest reduction in surface tension was recorded using de strain C1 with olive oil (10 mg/L) as a sole carbon sources. George and Jayachandran [25] conducted a research on rhamnolipid production from *Pseudomonas aeruginosa* MTCC 2297 and studied the cost-effectiveness of several waste material supplies (arrange peelings, carrot peel waste, lime peelings, coconut oil cake, and banana waste). The results reported that the orange peelings were the best substrate, producing 9.18 g/L of rhamnolipids with a surface tension reduction of up to 31.1 mN/m. Xia et al. [27] enhanced rhamnolipid production (80g/L) by *Pseudomonas aeruginosa* WJ-1using sunflower oil.

Corynebacterium lepusc an grow in glucose but if hexadecane is added it becomes able to synthesize large amounts of biosurfactants [13]. The way *Torulopsisbombicola* handles its carbon sources allows us to observe an increase in production of glycolipids [28]. Tuelva et al. [29] reported that *P. Putida* 21BN is able to produce rhamnolipids utilizing hexadecane carbon as its only source. A study done by Martínez-Toledo et al. [30] reported that *P. Putida* CB-100 was able to increase production of rhamnolipids when phenanthrene (200 mg/L) was added to the culture. Oliveira et al. [21] reported that *P. aeruginosa* FR produces biosurfactants when palm oil is used as a carbon source. Other studies have shown that *Pseudomonas* sp. Strain PP2, could produce biosurfactants growing on either phenanthrene or dextrose as carbon sources, but phenanthrene showed the highest production levels compared to dextrose [31]. de Lima, et al. [32] observed the rhamnolipid production due to *Pseudomonas aeruginosa* PACL growth on different waste frying soybean oils, where the maximum rhamnose production (4.3 g/L) was obtained using the NUSO soybean oil. *Pseudomonas aeruginosa* SP4 used palm oil as carbon source

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and produced biosurfactant at oil loading rates of 2 kg/m^3 24h, the surface tension of the liquid medium was reduced by 58% Pornsunthorntawee et al. [33]. *Pseudomonas* sp. LP1 produced biosurfactants in a culture where motor oil was the only available carbon source [34]. Rahman et al. [35] reported that *P. aeruginosa* DS10-129 produced 4.31 g/L of rhamnolipids in a mineral salts medium (MSM) with 2 g/L of glucose and soybean oil. Lima et al. [36] reported that *Bacillus subtilis* ICA56 produces a biosurfactant (120 mg/L) in mineral salts medium (MSM) with glycerol (20 g/L), which results in a reduction of the surface tension from 72 to 28 mN/m. In another report [37] a bacterial strain *Bacillus* sp. MTCC 5877 produces a biosurfactant in a MSM with 1% of glycerol added and 1% of cooking oil, the reduction in the surface tension was of 42 mN/m and the emulsification index (E_{24}) was 76%. A bacterium *Bacillus subtillus* R14914 produces 200 mg/L in a mineral medium with 20 g/l of sucrose Fernandes et al. [38]. *Bacillus tequilensis* ZB10 in a MSM containing hemicellulosic (50%) and cellulose (50%) hydrolysates yielded 1.52 g /L of biosurfactant that reduced surface tension to 38.6 mN/M. Hang el at. [39], tested a petroleum-degrader bacterium (*Bacillus* sp BS-8) at different concentrations in order to produce biosurfactant. Their results revealed that the best carbon source was glucose (20g/L) which reached the best oil displacement (62 mm). *Bacillus pseudomycoides* BS6 is a bacterium strain that was isolated from an oil-contaminated soil, with the capacity of producing biosurfactant that reduces surface tension from 76 mN/m to 30.2 mN/m, and produces an emulsion activity between 62.8% to 94.2% using waste soybean oil (20 g/L) as a carbon source. *Bacillus pumilus* 2IR is another bacterium strain which was isolated from an Iranian oil field as a potent biosurfactant-producing bacterium. The maximum biosurfactant production (30 nN/m) was observed in a medium formulated with 30.31 g/L of glucose, 0.8% of crude oil, as carbon sources.

Nitrogen and Phosphorus Sources

The effect of other macro nutrients added to the environment has been investigated as part of the production of biosurfactants. Apart from carbon (C), the most widely studied macro nutrients are Nitrogen (N) and Phosphorous (P). From these, N is of greater relevance. *Arthrobacterparaffineus* utilizes ammonium and urea salts as sources of nitrogen for the production of biosurfactants [20]. *P. aeruginosa* and *Rhodococcus*spp are able to use nitrates in order to increase the production of biosurfactants. A. *paraffineus* increases its production of biosurfactants when adding L-amino acids such as spastic acid, glutamic acid, asparagine and glycine to the environment. The structure of Surfact in produced by *Bacillus subtilis* modifies with concentrations of L-amino acids in the environment, producing Surfactin Val-7 or Leu-7. It has been observed, that Nitrate is the best source of nitrogen for the production of biosurfactants for *P. aeruginosa* strain 44T1 and for *Rhodococcus* strain ST-5 grown in olive oil and paraffin, respectively. Its production of biosurfactants started after 30 hours of growth, when the culture reached limiting concentrations of nitrogen and continued its production for up to 58 hours of fermentation. Patel and Desai (1997) introduced cornsteep liquor (0.5%, v/v) in the medium as a source of nitrogen to induce *P. aeruginosa* GS3 for the production of biosurfactants after 96 h of incubation. The produced rhamnolipid had the ability to emulsify various hydrocarbons (n-heptane, n-decane, n-hexadecane, benzene, toluene, n-paraffin, 2-methylnapthalene + n-hexadecane (1:1%, v/v, crude oil and olive oil). Xia et al. [27] used a culture medium with a N limit concentration (C/N of 8) and with $NaNO_3$ as a source of N; under these conditions in a fermenter (3000 L) with *Pseudomonas aeruginosa* WJ-1 50.2 g/L of rhamnolipids were produced and the surface tension decreased 43.09 mN/m.

In general, it has been observed that limiting concentrations of nitrogen favors the production of biosurfactants by various microorganisms. Davis et al. [40] observed that *Bacillus subtilis* ATCC 21332 increased its production of biosurfactants only when nitrate decreased in the culture medium. An increase in the production of rhamnolipids by *P. aeruginosa* LBI was observed following nitrogen depletion [19]. *Corynebacterium lepus* increased its production of biosurfactants when it used nitrate as its single source of nitrogen in the culture [41]. Some authors have observed that the synthesis of rhamnolipids by *P. aeruginosa* takes place when the source of nitrogen has run out and when its growth cycle is at its latency phase, and that when nitrogen sources are added production of rhamnolipids by *Pseudomona* ssp DSM 2874 [42] is inhibited. It has been reported that genes are activated to produce *P. aeruginosa* biosurfactants of *P. fluorescents* and *P. Putida* only under limited nitrogen conditions. Arino et al. [16] reported that glycolipid production by *Cellulomonascellulans* began after nitrogen sources (such as sodium nitrate) were exhausted in the medium and increased when their growth had stopped.

There is evidence of an existing connection between rhamnolipid synthesis and glutamine synthetase activity in *P. aeruginosa*. This enzyme delivers maximum activity levels when its exponential growth phase ends, and at that time its production of biosurfactants begins. Furthermore, Mulligan et al. [28] reported that the shift in metabolism to phosphate coincides with the production of biosurfactants. P limitation in the culture media was more effective for biosurfactant production by *P. aeruginosa* than N limitation, only when hexadecane or palmitic acid were used as carbon sources in anaerobic conditions [24]. In studies done by Martínez-Toledo and Rodríguez-Vázquez [43] it was also noted that *P. Putida* CB-100 is able to produce rhamnolipids with variations in its polar fraction due to the relationship changes among C:N:P, where glucose and phenanthrene were used as carbon sources in the culture medium.

Micronutrients

Several papers report on the great importance of micronutrients in the culture media for bacteria (Table 1). Guerra-Santos et al. [44] proved that under limited ion

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conditions Mg, Ca, K, Na, Fe and other trace elements, a high rate of biosurfactant production by *P. aeruginosa* is delivered. Various authors have shown that an overproduction of biosurfactants by *Pseudomonass*pp is obtained when it reaches its latency growth stage, under limited nitrogen and iron conditions.

Table 1: Speed agitation and pH tested in cultures in flasks for biosurfactant production by bacteria.

Microorganisms	Speed Agitation (rpm)	pH	Temperature (°C)	Reference
Bacillos subtilis ATCC 21332	300	7.0	27	Sheppard and Mulligan [58]
Pseudónimas aeruginosa U129791	200	6.5	37	Linhardt et al. [15]
Pseudomonas aeruginosa ATCC 9027	250		37	Mulligan et al. [59]
Bacillus subtilis ATCC 21332		6.7	30	Sheppard and Cooper [57]
Torulopsisbombicola ATCC 22214	220	5.6	30	Inoue and Ito
Pseudomonas aeruginosa UG2	200	6.8	28	Van Dike [61]
Pseudomonas aeruginosa DSM2659	133	6.8	37	Schenk et al. [56]
Pseudomonas aeruginosa GL1	200	7.3	30	Arino et al. [16]
Serratia marcescens MTCC 86	200			Pruthi and Cameotra [22] Arthrobacter
protophormiae MTCC 688	200			Pruthi and Cameotra [22]
Pseudomonas aeruginosa GS3	180	7.0	30	Patel and Desai [21]
Bacillus licheniformis mutant	200		42	Lin et al.
Cellulomonascellulans	200	7.3	30	Arino et al. [16]
Pseudomonas aeruginosa UG2	220	6.25	37	Mata-Sandoval et al. Mata-Sandoval et al.
Pseudomonas aeruginosa PG201	130		37	Beal and Betts [76]
Pseudomonas aeruginosa LB1	150	6.8	30	Benincasa et al. [19]
Pseudomonas aeruginosa ATCC 10145	300	6.2-7.0	28	Chayabutra et al. [24]
Pseudomonas aeruginosa DS10-129	200	7.5	30	Rahman et al. [35]
Pseudomonas aeruginosa KISR C1	200	7.5	35	Yateem et al. [25]
Pseudomonas aeruginosa KISR B1	200	6.8	35	
Pseudomonas putida	200			Pruthi and Cameotra [72]
Pseudomonas putida CB-100	200		28	Amézcua-Vega et al. [17]
Pseudomonas aeruginosa FR	150	7.0	29	Oliveira et al. [66]
Pseudomonas putida CB-100	150	7.0	37	Martínez-Toledo et al. [30]
Pseudomonas aeruginosa DHT2	200		50	Kumar et al. [48]
Pseudomonas aeruginosa PACL	550	7.0	30	De Lima et al. [32]

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Pseudomonas sp. LP1		7.2	29	Obayari et al.
Pseudomonas aeruginosa Pa24	120	7. 35	37	Bhat et al. [78]
Bacillus sp. MTCC 5977	120	7.0	30	Anjum et al. [37]
Bacillus subtilis R14914	200		30	Fernandes et al. [38]
Bacillus tequilensis ZSB10	150	7.4	35	Cortés-Camargo et al. [79]
Basillus pseudomycoides BS6	150	7.0	30	Li
*Bacullis pumilus*2IR	150	7.0	30	Fooladi et al. [68]

Phosphate, iron, magnesium and sodium are all elements of importance to the production of biosurfactants by *Rhodococcus*sp. but potassium and calcium are the elements of greatest importance. Iron also has an important impact on the production of rhamnolipids by *P. aeruginosa*. The concentration of manganese and iron affects the production of Surfactin by *Bacillus* subtilis. A report discusses how *B. subtilis* has an active transportation system for both ions, manganese may act as a cofactor to many enzymes involved in the metabolism of nitrogen, even in reactions from glutamate and ammonium catalyzed by the glutamine synthetase enzyme, this being its main assimilation mechanism of inorganic nitrogen, observed that with a concentration of 0.5 mg/L of $FeSO_4$ $7H_2O$ (C/Fe ratio of 72 400) in the culture medium rhamnolipids production by *Pseudomonas aeruginosa* increased (500mg/L). *Bacillus subtilis* ATCC 21332 increases its production of Surfactin in the presence of 1.7 mM of iron sulfate in the culture medium [45]. The iron concentration of 50-100 µg/L was the optimal concentration in the liquid medium for rhamnolipid production by *Pseudomonas aeruginosa* strain U129791 [38]. Amézcua-Vega et al. [17] observed an increase in the production of rhamnolipids by *P. Putida* CB-100 at a low C/Fe ratio (26000) in the culture medium, where the carbon source was corn oil at 2% (w/v). Similar results were observed with Rhodoccocussp. 51T7 that produced glycolipids (3g/L) at a low C/Fe ratio of 12000 [18].

Environmental Conditions

Environmental factors and growth conditions (Table 2) such as pH, temperature, agitation, availability of oxygen, etc., also affect the production of biosurfactants, via effects on both growth and cell activity. Production of rhamnolipids by *Pseudomonass*pp maximizes with a pH range between 6 and 6.5 and rapidly decreases when pH is higher than 7. It is also noted that the production of amphiphatic and disaccaride lipids by *N. corynbacteroides* does not undergo any changes when pH ranges from 6 to 8. It is known that surface tension as well as critical micelle concentration or CMC remain stable in a wide range of pH, however the emulsification has a limited pH range. Guerra-Santos et al. [44] determined that within a pH range from 6.2 to 6.4 *Pseudomonas aeruginosa* augments its production of rhamnolipids. On the other hand, *A. paraffinues* and the *Peudomona*sp strain DSM-2874, the temperature provokes an increase in biosurfactants production. A thermophilic *Bacillus* sp grew and produced a biosurfactant at temperatures above 40°C. Some biosurfactants do not alter either one of their properties: low surface tension or emulsifying efficiency, which remain stable even after sterilizing at 120°C for 15 minutes. Within a temperature range of 32 to 34°C *Pseudomona aeruginosa* increases production of rhamnolipids. Javaherin et al. [46] reported that *Bacillus licheniformis* produces biosurfactants at 50°C temperature. It has been observed that salt concentration affects production of biosurfactants which in turn have a direct effect over cell activity. Desai and Banat reported that salt concentrations above 10% (weight/volume) do not affect production of biosurfactants while a slight reduction in the CMC has been noted. Kumar et al. [47] reported that "*Planococcusmaitriensis* Anita I" (marine bacterium), produced biosurfactant in a medium with 0.5-12% of salt. A strain of *P. aeruginosa*was capable of growing in an environment which had a high degree of salinity (10%) together with high temperatures (45°C), using hexadecane as carbon source [48]. Elazzazzy reported that *VirgiBacillussalarius* KSA-T was the best bacterium in terms of biosurfactant production (30 mN/m) in a MSM with glucose (2%) and waste frying oil, urea and $NaNO_3$ were the best N sources in biosurfactant production (29.5 mN/m) by KSA-T (*V. salarius*), besides, this strain works at extreme conditions: pH 9, 45 -50°C and 4% of NaCl.

Table 2: Nutrient concentrations mostly used for biosurfactant production by bacteria.

Biosurfactant	Microorganism	Carbon source	N	P	Fe	Mg	Mn	Cu	Yield	Reference
			mol/L	mol/L	mol/L	mol/L	mol/L	mol/L	g/L	
Rhamnolipid	*P. aeruginosa* KY4025	n-Parafines (10%)	0.067	$5.2X10^{-4}$	$5.4x10^{-6}$	$1.8x10^{-5}$			8.5	Itoh
	P. aeruginosa DSM 2659	Glucose (18.2 g/L)	$3.7x10^{-3}$	0.057		$8.9x10^{-5}$	$4.8x10^{-3}$	$4.9x10^{-5}$	2.25	Schenk et al. [56]

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Rham nolipid	*P. aerug inosa* GL1	Glycerol (30 g /L)	0.17	0.321		$4x10^{-4}$			3.5	Arino et al. [16]
Rham nolipid	*P. aerug inosa* UW-1	Canola oil (6%)	0.437	0.05	$1x10^{-6}$	$2x10^{-3}$	$1x10^{-3}$	$1.4x10^{-3}$	24	Sim
Rham nolipid	*P. aer uginosa*	Glucose (18.2 g/L)	0.067	0.102	$1.8x10^{-6}$	$1.62x10^{-3}$	$8.8x10^{-3}$	$8.9x10^{-4}$	1.5	Guerra-Santos et al. [69]
Rham nolipid	*P. aerugi nosa* U129791	Corn oil (40 g/L)	0.329	0.1	$1x10^{-6}$	$2.0x10^{-3}$	$5x10^{-5}$	$1.2x10^{-4}$	5.4	Linhardt [15]
Rham nolipid	*P. aerug inosa* ATCC 10145	Hexadecane (8%)	0.018	0.0630.32	$1.1x10^{-4}$	$1.2x10^{-3}$	$5.9x10^{-5}$		0.0125 g/g CDW	Chayabutra and Ju [24]
Rham nolipid	*P. aerug inosa* AT10	Waste free fatty a 2cids (5%) and ERASOL food oil refinery	0.135	0.03	$3.9x10^{-5}$	$2.0x10^{-3}$			9.5	Abalos et al. [74]
Rham nolipid	P.aerug inosa LBI	Soapstock (30g/L)	0.108	0.009	3.6x10-6	0.002	$2.5x10^{-5}$	$2.5x10^{-5}$	12	Benincasa et al. [19]
Liche nsyn	*Bacillus lichenif ormis* R2	Glucose (34g/L)	0.013	0.082	$5.9x10^{-6}$	$4.0x10^{-5}$	$8.9x10^{-6}$			Joshi et al. [6]
Biosurf actant	*Bacillus* sp.	Glucose (30g/L)	0.075	0.006	$2x10^{-4}$	$1.2x10^{-3}$			2.42	Mukherjee et al. [7]
Biosurf actant	*Bacillus* pumilus DSVP18	Potato peels (20g/L)	0.05	0.039	$3.6x10^{-5}$	$7.2x10^{-5}$			3.2	Sharma et al. [54]
Biosurf actant	*VirgiBa cillussa larius* KSA-T	Glucose (20g/L)	0.094	0.048	$3.6x10^{-5}$	$2.4x10^{-3}$	$2.2x10^{-5}$	$6.3x10^{-5}$		Elazzazy et al. [80]
Rham nolipid	*P. aerug inosa* Pa24	Glycerol (30g/L)	0.034	0.01	$3.0x10^{-3}$	$2x10^{-3}$	$1.1x10^{-3}$	$1.4x10^{-4}$	2.36	Bhat et al. [78]
Biosurf actant	*Bacillus* sp. MTCC5877	Glycerol (1%)	0.025	0.013	$3x10^{-4}$	$8x10^{-4}$				Anjum et al. [37]
		Cooking oil (1%)								
Biosurf actant	*Bacillus subtilis* R14914	Sucrose (20g/L)	0.05	0.08	$1.8x10^{-5}$	$1.25x10^{-3}$	$1.6x10^{-4}$	$2.9x10^{-5}$	0.2	Fernandes et al. [38]
Biosurf actant	*Bacillus tequil ensis* ZB10	Hemicel lulosic (50 %) and Cellulosic (50%) hydrol ysates	0.08	0.17		$1.6x10^{-3}$			1.52	Cortés-Camargo et al. [79]
Biosurf actant	*Bacillus* sp BS-8	Glucose (20g/L)	0.372	0.028		0.002				Chang et al. [39]
Biosurf actant	*Bacillus Pseudom ycoides* BS6	Soybean oil (20g/L)	0.607	7.3x10-3		$2x10^{-3}$				Li
Biosurf actant	*PeniBacil lusdendrit iformis* CN5	Pyrene (0.1g/L)	0.045	0.087	$1.1x10^{-4}$	$1.6x10^{-3}$	$1.1x10^{-6}$	$1.8x10^{-6}$	6	Bezza and Chirwa [77]

Biosurfactant producers are mainly aerobic microorganisms that grow in liquid media, which is why aeration plays an important role in the biosurfactant yield (Table 2). The production of biosurfactants by *Nocardiaerythopolis* decreases while agitation speed increases in the culture environment. It has been reported that in order for some yeasts to produce biosurfactant, performance is enhanced at both high agitation speeds and a high degree of aeration. A paper reports that the transference of oxygen is an important parameter in optimization processes for large scale production of Surfactin by *B. subtilis*. For

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the strain *P. aeruginosa* PACL, aeration was the principal variable that affected their biosurfactant production, and the maximum rhamnose concentration (3.3g/L) was obtained at 300 rpm.

Perspectives

Production costs are often an obstacle for biotechnological processes, particularly those involving biosurfactants. Success in the production of biosurfactants hinges on the development of economic processes and on the use of low cost raw materials which represents between 10 and 30% of production costs. Biosurfactants must compete with surfactants of petrochemical origin in three key areas: costs, functionality and production capacity. High production costs m ay be afforded on a low-demand biosurfactant or on products of high commercial value such as cosmetics, medicines, etc. However, for applications aimed at the recovery of contaminated soils in which large volumes of biosurfactants are needed, its high production cost is incompatible with its use. Makkar and Cameotra [49] have suggested four factors which may cut back production costs. Microorganisms (choose, adapt or modify to obtain peak production performance), the process (choose, adapt or design for low capital investments and operating costs), the substrate for microorganisms and the feeding processes (choose to reduce costs), and the sub-products from the production process (minimize residue) [50-58].

Some research on the choice of adequate substrates has focused mostly on tropical agro-industrial plantations and on residues [59-65]. These include plantations like tapioca, soy, sugar beet, sweet-potato, potato, tender sorghum, harvest residues like bran, straw, wheat and rice, husk of soybean, corn and rice, sugarcane bagasse, and tapioca; residues from the coffee industry such as coffee pulp, coffee grain husks, residual ground coffee; residues from fruit processing like grapefruit, apple and grapes; residues from pineapple and carrot processing, banana residues, residues from the processing of milling to obtain oil, such as coconut paste, soy paste, peanut paste, canola, palm, and others such as the shavings from corn cobs, sheath of carob trees, tea residues, etc [65-74]. Additional substrates have been suggested for the production of biosurfactants such as water miscible residues, molasses, milk serum, or the residual distiller. Therefore, it is very important to select the microorganisms and know their different metabolic capabilities, in order for them to be used as potential tools in the bioremediation of polluted matrixes [75-80]. In addition, saving costs and likely reducing the time of the biological process, as well as their potential applications in environmental restauration, food, cosmetics, agriculture and pharmaceutical industries.

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