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Biodegradability of bacterial surfactants

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Abstract This work aimed at evaluating the biodegradability of different bacterial surfactants in liquid medium and in soil microcosms. The biodegradability of biosurfactants by pure and mixed bacterial cultures was evaluated through CO_2 evolution. Three bacterial strains, *Acinetobacter baumanni* LBBMA ES11, *Acinetobacter haemolyticus* LBBMA 53 and *Pseudomonas* sp. LBBMA 101B, used the biosurfactants produced by *Bacillus* sp. LBBMA 111A (mixed lipopeptide), *Bacillus subtilis* LBBMA 155 (lipopeptide), *Flavobacterium* sp. LBBMA 168 (mixture of flavolipids), *Dietzia Maris* LBBMA 191(glycolipid) and *Arthrobacter oxydans* LBBMA 201(lipopeptide) as carbon sources in minimal medium. The synthetic surfactant sodium dodecyl sulfate (SDS) was also mineralized by these microorganisms, but at a lower rate. CO_2 emitted by a mixed bacterial culture in soil microcosms with biosurfactants was higher than in the microcosm containing SDS. Biosurfactant mineralization in soil was confirmed by the increase in surface tension of the soil aqueous extracts after incubation with the mixed bacterial culture. It can be concluded that, in terms of biodegradability and environmental security, these compounds are more suitable for applications in remediation technologies in comparison to synthetic surfactants. However, more information is needed on structure of biosurfactants, their interaction with soil and contaminants and scale up and cost for biosurfactant production.

Keywords Biosurfactant · Biodegradation · Respirometry · Mixed culture · SDS

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Introduction

Biological degradation of surfactants is the most important mechanism for irreversible removal of these substances from aquatic and terrestrial environments and, hence, biodegradability is considered an important feature when evaluating environmental risk associated with surfactant use (Berna et al. 2007). The biodegradation of surface active molecules occurs when microorganisms use these compounds as carbon and energy sources and involves two steps. In the first step, the hydrocarbon chain is broken, leading to structural modification and immediate loss of amphiphilicity. In the second step, the products resulting from the primary degradation are transformed into CO_2, water and minerals (Garcia et al. 2006).

Biodegradability of a surfactant may have both positive and negative effects on its use in bioremediation processes (Li and Chen 2009). The negative effects include the depletion of minerals and oxygen, the potential toxicity of intermediaries in the degradation pathway (Tiehm 1994) or the preferential degradation of the surfactant over that of the pollutant (Li and Bai 2005). The most obvious positive effect of the degradation of a surfactant is its removal from the environment in which it was used.

While many surfactants can be degraded by microorganisms, some synthetic surfactants used in remediation, such as linear alkylbenzene sulfonate (LAS), are persistent under anaerobic conditions (Garcia et al. 2006). It was recently shown that a bacterial surfactant, rhamnolipid, was biodegradable under aerobic, nitrate reducing, sulphate reducing and fermentation conditions, while a synthetic surfactant (Triton X-100) was only partially biodegradable under aerobic conditions and non-biodegradable under all other tested conditions (Mohan et al. 2006). The test was accomplished in accordance with Organization for Economic Cooperation and Development (OECD) 301D for ready biodegradability.

Biosurfactants are generally considered to be less toxic and more biodegradable than synthetic surfactants. There is, however, little data available in the literature on biosurfactant biodegradation (Frank et al. 2010). This work was therefore undertaken to evaluate the biodegradability of biosurfactants produced by different bacterial species in the liquid phase and in soil under aerobic conditions.

Methods

Surfactants

Six surfactants were used in this study, one synthetic (SDS, Merck) and five biological surfactants produced by the bacterial strains *Bacillus* sp. LBBMA 111A (mixed lipopeptide), *B. subtilis* LBBMA 155 (lipopeptide), *Flavobacterium* sp. LBBMA 168 (mixture of flavolipids), *Dietzia maris* LBBMA 191 (glycolipid), and *Arthrobacter oxydans* LBBMA 201 (lipopeptide). The biosurfactants were produced in the Laboratory of Biotechnology and Biodiversity for the Environment (LBBMA) of the Department of Microbiology, Federal University of Viçosa (Brazil) and partially purified using acid precipitation, ultrafiltration and thin-layer chromatography (TLC) techniques. The surface active properties of each surfactant are described in Table 1.

Surfactin, iturin and fengycin of *Bacillus* sp. LBBMA 111A, surfactin of *B. subtilis* LBBMA 155, glycolipid of *D.Maris* LBBMA 191 and Arthrofactin of *A. oxydans* LBBMA 201 were produced in mineral medium supplemented with 2% glucose (w v^{-1}) and 2% hexadecane (v v^{-1}) (added after glucose exhaustion). Flavolipids of *Flavobacterium* sp. LBBMA 168 were produced in drop-collapse mineral medium (Bodour and Miller-Maier 1998) containing 2% glucose (w v^{-1}) as sole carbon source. Cultures were grown in flasks at 30°C in an orbital shaker (New Brunswick Scientific) under constant agitation (200 rpm) for 7 days.

Table 1 Critical micelle concentration (CMC), surface tension at CMC (γ_{CMC}), and correlation equations between surface tension (γ) and concentration (C) of surfactants used in this study

Surfactant type	Abbreviations	γ_{CMC} (mN m^{-1})	CMC (mg L^{-1})	Equation
Lipopeptides	LBBMA 111A	33.9	150	$\gamma = 26.53^{**}e^{(94.28^{*}/(C + 79.6^{*}))}$
Lipopeptide	LBBMA 155	34.6	180	$\gamma = 27.69^{**}e^{86.04^{*}/(C + 77.1^{*}))}$
Flavolipids	LBBMA 168	38.8	200	$\gamma = 37.98–2290.15^{o}/C + 599127.5^{**}/C^2–22386626^{**}/C^3$
Glycolipid	LBBMA 191	35.0	150	$\gamma = 29.75^{**}e^{(52.2^{*}/(C + 45.76^{*}))}$
Lipopeptide	LBBMA 201	36.6	200	$\gamma = 27.25^{**}e^{(118.18^{*}/(C + 110.08^{*}))}$
Sodium dodecyl sulfate	SDS	39.0	2120	$\gamma = 29.26^{**}e^{(397.4^{**}/(C - 703.13^{**}))}$

LBBMA 111A surfactant produced by *Bacillus* sp. LBBMA 111A, *LBBMA 155* surfactant produced by *Bacillus subtilis* LBBMA 155, *LBBMA 168* surfactant produced by *Flavobacterium* sp. LBBMA 168, *LBBMA 191* surfactant produced by *Dietzia maris* LBBMA 191, *LBBMA 201* surfactant produced by *Arthrobacter oxydans* LBBMA 201, *SDS* synthetic surfactant sodium dodecyl sulfate

Significant at °10%, *5% and **1% probability

Isolation and purification of biosurfactants were performed after removal of cells by centrifugation (12,000×*g*, 20 min, 25°C), followed by filtration using a 0.45 μm pore size membrane filter. Biosurfactants LBBMA 111A, LBBMA 155 and LBBMA 201 were acid-precipitated, by adjusting the pH of the medium to 2.0 with 12 M HCl. Biosurfactant pellets were recovered by centrifugation (12,000×g, 20 min, 20°C) (Vater et al. 2002) and dissolved in water at pH 7.0. The products were extracted with chloroform and methanol, filtered with qualitative filter paper, and, after solvent evaporation, submitted to TLC on silica gel 60 F254 (VETEC) plates and quantified. Biosurfactant LBBMA 168 was recovered using an ultrafiltration cell (Amicon model) equipped with 500 and 1000 Da molecular weight cutoff membranes (Lin et al. 1998), followed by TLC and quantification. The compounds plotted on the TLC plates were visualized under ultraviolet light (UV 254 nm) and revealed by ninhydrin (0.2%, w v^{-1}) (Riedel–de–Haen, Seelze, Germany) and 3 M H_2SO_4, followed by heating the plates at 100°C for 5 min (Horowitz et al. 1990). The impure fractions were separated by preparative TLC on glass plates (20 × 20 mm^2) pre-coated with silica gel 60 F_{254} (VETEC) and eluted in mixtures of chloroform and methanol. The bands on the TLC plates were detected under UV light (254 nm), and those corresponding to the compounds of interest were scraped from the plates, pooled, and extracted three times with chloroform.

The surfactant solutions were prepared in ultrapure water (milliQ_{plus}, MILLIPORE), adjusted to pH 6.8 with 1 M NaOH. Surfactant concentrations used in the degradation trials were equivalent to 2 times their critical micelle concentration (2CMC) (Table 1).

Microorganisms used in degradation tests

Pure cultures of *Acinetobacter haemolyticus* LBBMA 53, *Acinetobacter baumanni* LBBMA ES11, and *Pseudomonas* sp. LBBMA 101B were used in the study of surfactant biodegradation in liquid medium. The evaluation of surfactant degradability in soil microcosms was conducted with a mixed culture containing the bacterial strains *Acinetobacter baumannii* LBBMA 04, *Acinetobacter junni* LBBMA 36, *Pseudomonas* sp. LBBMA 101B and *Acinetobacter baumanni* LBBMA ES11. All bacterial strains were isolated from hydrocarbon-contaminated soil and water and were previously shown to degrade different petroleum hydrocarbons.

Study of surfactants biodegradation

Biodegradability test in liquid medium

The degradability of biosurfactants in liquid medium by pure cultures of bacteria was determined using a respirometry technique (Franzetti et al. 2006). A volume of inoculum sufficient to obtain a final OD_{600} of 0.05 was added to 125 ml respirometric bottles (GibcoBRL, Life Technologies) containing 10 ml of a solution of biosurfactant (at 2CMC) in NMP mineral medium (Margesin and Schinner 1997). The bottles were attached to a respirometer with an infrared (IR) detector (Sable Systems International, NE, USA) and the CO_2 produced by bacterial cells was recorded over seven days. The control was performed by omitting the biosurfactants from the growth medium.

At the end of the incubation period, the residual biosurfactant concentration in solution was determined after removing the cells by centrifugation at 10,000×g for 20 min. The surface tension of the supernatant was measured using a du Nouy ring tensiometer (Fisher Scientific, Pittsburgh, USA) (Cooper et al. 1979).

Biodegradability test in soil microcosms

Soil used in this study was collected from 0 to 20 cm deep layer in Viçosa (Minas Gerais, Brazil) and was classified as Haplustox (Soil Survey Staff 1999). Its physical and chemical characterization was carried out in the Soil Department Routine Analysis Laboratory of the Federal University of Viçosa. The organic carbon content was quantified by wet oxidation of organic matter, using potassium dichromate solution in acid medium (Yeomans and Bremner 1988). The cation exchange capacity (CEC) was determined by measurement of Ca^{2+}, Mg^{2+} and Al^{3+} desorbed in the soil, after saturation with a solution of 0.1 M $BaCl_2$ (Hendershot et al.1993). This soil presented high clay content, average organic matter content and high acidity (Table 2). Clayey soils tend to retain contaminants like heavy metals and PAH's more strongly, which makes their removal by the physical and chemical treatments currently in use more difficult

Table 2 Physical–chemical characterization of the experimental soil

Parameter	Value
Thick sandy (%)	18.00
Fine sand (%)	17.00
Silt (%)	3.00
Clay (%)	62.00
pH (in H_2O)	4.30
Al^{3+} ($cmol_c$ dm^{-3})	1.24
Ca^{2+} ($cmol_c$ dm^{-3})	0.10
Mg^{2+} ($cmol_c$ dm^{-3})	0.10
Organic matter (dag kg^{-1})	3.32
P (Mehlich-1) (mg dm^{-3})	2.00
K (Mehlich-1) (mg dm^{-3})	29.00
P rem (mg L^{-1})[a]	11.00

[a] Phosphorus remaining in mg l^{-1} obtained by the agitation of 60 mg l^{-1} of P in a solution of $CaCl_2$ 10 mmol with the soil sample in a reaction soil:solution 1:10 for 1 h

(Dennis et al. 1992). In addition to clay, the organic matter content is also associated with the retention of contaminants (Mulligan et al. 2001).

For each bacterial strain in the mixed culture, a volume of inocula sufficient to obtain a final concentration of 10^6 CFU g^{-1} of soil was added to 125 ml respirometric bottles (GibcoBRL, Life Technologies). The bottles contained 10 g of dry sieved (2 mm) soil sterilized by gamma radiation. Soil humidity was adjusted to 60% of the maximum soil retention capacity by adding the surfactant solutions at 2CMC. The bottles were attached to the respirometer and the CO_2 produced by bacterial cells was recorded over 166 h. Controls were done by omitting the biosurfactants or the inoculums.

At the end of the incubation period, residual biosurfactant was recovered by adding 25 ml of distilled water to the bottles, followed by shaking at 200 rpm for one hour and centrifugation at 10,000×g for 20 min. The supernatant was filtered through a Millipore membrane (0.45 μm), and its surface tension measured by the du Nouy ring method in a tensiometer (Fisher Scientific, Pittsburgh, USA) (Cooper et al. 1979). The values of surface tension were used to calculate the concentration of surfactant expressed as CMC, using previously constructed regression equations of surfactant concentration versus surface tension (Table 1). The concentration of each surfactant in the soil was estimated at the beginning and at the end of the incubation period. The reduction in the concentration of each surfactant was calculated and expressed as percentage of the CMC.

Results and discussion

Potential for degradation of biosurfactants by pure bacterial cultures

Acinetobacter haemolyticus LBBMA 53, *A. baumanni* LBBMA ES11 and *Pseudomonas* sp. LBBMA 101B were able to degrade all surfactants evaluated when grown in liquid medium (Table 3). *A. haemolyticus* LBBMA 53 and *A. baumanni* LBBMA ES11 did not differ in their capacity to degrade the biosurfactants nor the synthetic surfactant SDS. The ability of *Pseudomonas* sp. LBBMA 101B to degrade biosurfactants was about 54 times higher than that of *A. baumanni* LBBMA ES11 and 60 times higher than that of *A. haemolyticus* LBBMA 53. The ability of *Pseudomonas* sp. LBBMA 101B to degrade the synthetic surfactant SDS was also higher than of *A. baumanni* LBBMA ES11 (12 times) and *A. haemolyticus* LBBMA 53 (9 times) (Table 3).

There was no significant difference in the intensity of degradation between the biosurfactants, nor between the intensity of degradation of the biosurfactants LBBMA 111A, LBBMA 155, LBBMA 168, LBBMA 201 and of the synthetic surfactant SDS by *A. haemolyticus* LBBMA 53 and *A. baumanni* LBBMA ES11 (Table 3). On the other hand, respiratory activity by these strains in samples with the trehalolipid surfactant LBBMA 191 produced by *D. maris* was significantly higher than in samples with SDS.

The intensity of SDS degradation was significantly lower (about 10 times) than that of biosurfactant degradation by *Pseudomonas* sp. LBBMA 101B. This effect may be related to the toxicity of the surfactant or of an intermediate formed during its degradation. According to Van Ginkel (1996), biodegradation of some synthetic surfactants leads to the generation of toxic metabolites. When evaluating the degradation of SDS by pure cultures, Singh et al. (1998) reported that the growth of *Pseudomonas aeruginosa* was completely inhibited by the presence of SDS, even at concentrations below its CMC.

Table 3 Production of CO_2 by *Acinetobacter haemolyticus* LBBMA 53, *A. baumanni* LBBMA ES11 and *Pseudomonas* sp. LBBMA 101B in mineral medium with biosurfactants or with the synthetic surfactant SDS

Treatment[a]	CO_2 (μmol ml^{-1})[b]		
	A. haemolyticus LBBMA 53	*A. baumanni* LBBMA ES11	*Pseudomonas* sp. LBBMA 101B
LBBMA 111A	3.31 ABb	3.99 ABb	226.71 A
LBBMA 155	3.15 ABb	4.22 ABb	233.67 A
LBBMA 168	3.24 ABb	4.04 ABb	208.19 A
LBBMA 191	5.12 Ab	4.40 Ab	209.15 A
LBBMA 201	3.68 ABb	4.09 ABb	234.73 A
SDS	2.86 Bb	2.09 Bb	24.78 B
Control	0.16 Ca	0.12 Ca	2.00 C

[a] Biosurfactants produced by *Bacillus* sp. LBBMA 111A; *B. subtilis* LBBMA 155; *Flavobacterium* sp. LBBMA 168; *Dietzia maris* LBBMA 191 and *Arthrobacter oxydans* LBBMA 201. *SDS* synthetic surfactant sodium dodecyl sulfate. The data represent the average of two replicates. The variability among the replicates within any treatment measurement timepoint was less than 10%. Averages followed by the same capital letter, within a column, and small letters, across a line, do not differ significantly (Tukey's test at 5% of probability)

[b] Data refer to CO_2 accumulated over 166 h of static incubation at 30°C. Initial concentration of surfactants was equivalent to 2CMC

In recent work conducted in our lab, the effect of different concentrations of SDS (2, 4 and 8CMC) on the growth of strains *A. baumanni* LBBMA ES11 and *Pseudomonas* sp. LBBMA 101B in mineral medium supplemented with glucose was evaluated. Bacterial growth was partially inhibited in presence of SDS at a concentration of 2CMC and growth was totally inhibited when SDS was present at concentrations of 4 and 8CMC, confirming the toxic effect of this surfactant.

Respiratory activity in the presence of surfactants was significantly higher than in inoculated mineral medium alone (Table 3). This result indicates that respiratory activity was related to the use of surfactants in respiratory metabolism, and not the consumption of cellular reserves (endogenous metabolism). Although the confirmation that the CO_2 emitted originated from degradation of surfactant molecules requires the use of other techniques, such as ^{14}C-labelling of surfactants, the higher respiratory activity in the presence of surfactants is an indication that these compounds were effectively the source of CO_2 emissions.

The increase of surface tension of the medium at the end of the incubation period (Table 4) confirms that the surfactants were metabolized and partially converted to CO_2. The surfactants were added to the medium at a concentration equivalent to 2CMC. When the concentration of surfactants in an aqueous solution reaches values above its CMC, most of the molecules form micelles, and the surface tension value does not change with a further increase in surfactant concentration (Rosen 2004). However, as the concentration decreases below the CMC, the surface tension increases up to a limiting value which, in pure water at 25°C, is about 72 mN m^{-1} (Rosen 2004). It follows therefore that the elevation of the surface tension of the medium after incubation is an unequivocal indication that surfactant concentrations dropped to below their CMC.

The surface tension in culture medium inoculated with *Pseudomonas* sp. LBBMA 101B was significantly higher than in media inoculated with isolates *A. haemolyticus* LBBMA 53 or *A. baumanni* LBBMA ES11 (Table 4), signifying a lower concentration of surfactant. This result is consistent with the higher respiratory activity of samples inoculated with *Pseudomonas* sp. LBBMA 101B (Table 3), and again suggests that the respiratory activity was linked to the degradation of surfactants. Surface tensions in media inoculated with *A. baumannii* LBBMA ES11 were significantly higher than in media inoculated with *A. haemolyticus* LBBMA 53, except in the presence of the biosurfactant produced by *D. maris* LBBMA 191 (Table 4). Although statistically different, average surface tension values in media inoculated with these two strains were very similar. For example, the difference between the surface tension in the media containing biosurfactant LBBMA 111A and inoculated with *A. haemoliticus* or *A. baumannii* was 1.1 mN m^{-1}. This difference represents a variation of

Table 4 Surface tension (γ) in supernatants of surfactant-containing culture medium inoculated *with Acinetobacter haemolyticus* LBBMA 53, *A. baumanni* LBBMA ES11 or *Pseudomonas* sp. LBBMA 101B, after cultivation for 166 h at 30°C

Treatment[a]/(γ Initial)[b]	γ_{Final} (mN m^{-1})		
	A. haemolyticus LBBMA 53	*A. baumanni* LBBMA ES11	*Pseudomonas* sp. LBBMA 101B
LBBMA 111A/32.7	47.7 C	48.8 B	60.6 A
LBBMA 155/33.1	42.6 C	46.2 B	62.6 A
LBBMA 168/34.2	45.7 C	47.6 B	62.3 A
LBBMA 191/34.4	50.6 B	50.9 B	61.2 A
LBBMA 201/36.5	49.9 C	51.2 B	64.1 A
SDS/37.9	42.4 C	49.0 B	52.6 A

Averages followed the same letter in each line do not significantly differ (Tukey's test at 5% of probability)

[a] Biosurfactants produced by *Bacillus* sp. LBBMA 111A; *B. subtilis* LBBMA 155; *Flavobacterium* sp. LBBMA 168; *Dietzia maris* LBBMA 191 and *Arthrobacter oxydans* LBBMA 201. *SDS* synthetic surfactant sodium dodecyl sulfate. Each data point is the average of two replicates. The variability among the replicates within any treatment measurement timepoint was less than 10%

[b] Surface tension of culture medium, at the start of cultivation. The values correspond to surface tension at the CMC

only 2% of the initial ST, and is consistent with the observation that there was no significant difference between the respiratory activities of these two strains (Table 3).

Biosurfactant degradation by mixed bacterial culture in soil

Biosurfactants stimulated CO_2 emissions by the mixed bacterial culture in soil microcosms (Table 5).

There were no significant differences between the respiratory activities in microcosms that received different biosurfactants (Table 5). This result is consistent with those obtained in the tests conducted in mineral medium inoculated with pure cultures (Table 3), and indicates that the biosurfactants are also biodegradable in soil.

CO_2 emitted from the soil microcosms with biosurfactants was significantly higher than from the microcosm with the synthetic surfactant SDS (Table 5). Significant differences between the emission of CO_2 in the treatments with biosurfactants and with SDS in mineral medium, were only consistently found in those inoculated with *Pseudomonas* sp. LBBMA 101B (Table 3). It was also shown that the emission of CO_2 in the medium inoculated with *Pseudomonas* was almost two orders of magnitude greater than that obtained with the two *Acinetobacter* strains. Based on data obtained in liquid medium, it is speculated that most of the CO_2 emitted by soil microcosms originated from *Pseudomonas* sp. LBBMA 101B activity, a component of the mixed

Table 5 CO_2 emission and surface tension (γ) in sterile soil microcosms and microcosms inoculated with a mixed bacterial culture, in the presence of surfactants, after 166 h of incubation at 30°C

Microcosm[a]	CO_2 emitted (μmol g^{-1})	γ (mN m^{-1})		Reduction of surfactant concentration (%)
		γ Initial[b]	γ Final	
LBBMA 111A	2.31 A	37.9	54.3	68.3 A
LBBMA 155	2.16 A	36.7	52.6	69.1 A
LBBMA 168	2.15 A	37.3	51.4	42.5 C
LBBMA 191	1.97 A	37.7	52.6	59.6 B
LBBMA 201	1.49 A	36.8	51.2	73.4 A
SDS	0.63 B	39.5	48.6	24.8 D
Control (water)	0.18 B	–	–	-

[a] Biosurfactants produced by *Bacillus* sp. LBBMA 111A; *B. subtilis* LBBMA 155; *Flavobacterium* sp. LBBMA 168; *Dietzia maris* LBBMA 191 and *Arthrobacter oxydans* LBBMA 201. *SDS* synthetic surfactant sodium dodecyl sulfate. The data represent the average of two replicates. The variability among the replicates within any treatment measurement timepoint was less than 10%. Average followed the same letter, within a column, do not differ (Tukey's test at 5% of probability)

[b] Surface tension of soil solution, at the start of cultivation. The values correspond to surface tension at the CMC

culture used as inoculum. The (calculated) surface tension values at the end of the incubation period in the soil extracts containing surfactants were only slightly higher than the surface tension values at the beginning of incubation (Table 5). This increase was much less significant than that observed in liquid

medium, indicating a higher residual surfactant concentration in the soil microcosms. This result suggests that both biosurfactants and the synthetic surfactant SDS were adsorbed onto the soil particles, making them more resistant to degradation by microbial populations. The lowest decrease in surfactant concentration was observed in soil with SDS, which is consistent with the lower respiratory activity in this treatment and points to the low biodegradation of the synthetic surfactant in the soil (24.8%). The highest surface tension values were obtained in soil solutions containing biosurfactants, and corresponded to degradations of 68.3% of biosurfactant LBBMA 111A, 69.1% of biosurfactant LBBMA 155, 42.5% of biosurfactant LBBMA 168, 59.6% of biosurfactant LBBMA 191 and 73.4% of biosurfactant LBBMA 201 by the mixed culture.

The surfactants used in this study are classified as high molecular weight anionic surfactants. The degradation process of these compounds depends on their structure but initiates with the cleavage of the hydrophobic surfactant chain, leading to an increase in the CMC. This structural modification of the surfactant alters its surface active properties and reduces some of its undesirable effects in the environment, such as foam formation. In the second stage, the products resulting from the primary degradation are transformed into CO_2, water and minerals, representing the complete degradation of surfactant (mineralization). Degradation of the hydrophobic alkyl chain begins with oxidation of the terminal methyl group (ω-oxidation) to an alcohol, an aldehyde and finally a carboxylic acid. The carboxylic acid undergoes β-oxidation, catalyzed by alkane monooxygenases and dehydrogenases. This is a predominant aerobic degradation mechanism in nature (Schleheck 2003).

While the biodegradation of any compound in the environment is desirable from the standpoint of environmental safety, very rapid degradation can limit its potential for some applications. Surfactants, including those of biological origin, are widely used in industrial and environmental applications (Nitschke and Pastore 2002). Among the most important environmental applications are their role in dispersing oil spills in aquatic environments, as components of washing solutions for solid wastes (including soil) contaminated with hydrophobic organic pollutants and heavy metals and in bioremediation, with the aim of increasing the solubility and bioavailability of hydrophobic molecules for microbial degradation (Desai and Banat 1997; Singh et al. 2007). In such cases, surfactants must exhibit sufficient stability to perform the activity for which they were introduced. In this context, the biosurfactants evaluated in this study presented a high potential for environmental applications, since they combined a reasonable degree of stability with the possibility of being degraded by microbial populations, both in liquid medium and in soil.

Conclusions

The biosurfactants surfactin, iturin and fengycin produced by *Bacillus* sp. LBBMA 111A, surfactin produced by *B. subtilis* LBBMA 155, glycolipid produced by *D. maris* LBBMA 191, arthrofactin produced by *A. oxydans* LBBMA 201 and flavolipids produced by *Flavobacterium* sp. LBBMA 168 are used as substrates by pure cultures of *A. haemolyticus* LBBMA 53, *A. baumanni* LBBMA ES11 and *Pseudomonas* sp. LBBMA 101B in mineral medium. In liquid medium, *Pseudomonas* sp. LBBMA 101B had a biosurfactant degradation capacity about two orders of magnitude larger than *A. haemolyticus* LBBMA 53 and *A. baumanni* LBBMA ES11. The biodegradation of the synthetic surfactant SDS in liquid medium by *A. haemolyticus* LBBMA 53 and *A. baumanni* LBBMA ES11 did not differ from that of the biosurfactants, but was an order of magnitude smaller than biosurfactant degradation by *Pseudomonas* sp. LBBMA 101B. In soil microcosms, a mixed culture composed of *A. baumannii* LBBMA 04, *A. junni* LBBMA 36, *Pseudomonas* sp. LBBMA 101B and *A. baumanni* LBBMA ES11 was able to use the biosurfactants as substrates. The biosurfactants did not differ in their ability to stimulate the respiratory activity of the mixed culture, but there was no significant degradation of the synthetic surfactant SDS in soil inoculated with the mixed culture, during seven days of incubation. In a comparative analysis, the biological surfactants had a higher degradation potential than the synthetic surfactant SDS. They exhibited stability in soil compatible with environmental applications, while at the same time had a degradation capacity that would reduce the risk of their accumulation in the environment. It can be concluded that, in terms of biodegradability and

environmental security, these compounds are more suitable for environmental applications than the synthetic surfactant SDS.

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