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Biosurfactant production from industrial wastes with potential remove of insoluble paint

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ABSTRACT

Biosurfactants are amphipathic compounds formed by a hydrophilic and hydrophobic component, this characteristic confers to these compounds the possibility of several applications. The aim of this study was to produce biosurfactants from different industrials wastes using *Corynebacterium aquaticum* and *Corynebacterium* spp. CCT 1968 and study the application in paint removal. The production of biosurfactants was evaluated through surface tension, emulsifying activity and character ionic. The analyses were carried out at 0, 24, 48 h. The biosurfactants that presented lower surface tension and higher emulsifying activity were applied in insoluble paint. The microorganism *Corynebacterium aquaticum* showed efficient biosurfactant production when using fish and bagasse residues as carbon source. The surface tension obtained for these treatments was 27.8 and 33.9 mN/m and emulsifying capacity was 87.6 and 61.6%, respectively. The *Corynebacterium* spp. CCT 1968 produced biosurfactants only in the medium containing fish waste (28.5 mN/m). The biosurfactants produced by both microorganisms showed anionic character. The applied biosurfactants showed potential use in solubilization and paint removal. Therefore, the residues of fish and sugarcane bagasse showed efficient as carbon sources to obtain biosurfactants. In addition, the preliminary paint removal application presented great results that can be explored in the future.

1. Introduction

Biosurfactants are surface-active compounds synthesized by microorganisms, which have the capacity to reduce surface and interfacial tensions of solutions (Franzetti et al., 2011). They are an alternative for the replacement of chemical surfactants produced from petroleum (Nitschke et al., 2004; Luna et al., 2012; Ismail et al., 2013). These compounds are produced by a wide variety of bacteria and fungi. Thus, several types of structures are formed as phospholipids, glycolipids, lipopeptides, polymeric surfactants and others. Depending on the structure of biosurfactant the interaction with pollutants could be different (Sriram et al., 2011).

Biosurfactants have several advantages over the surfactants chemically produced, such as lower toxicity, higher biodegradability, improved environmental compatibility, higher selectivity and specific activity in conditions of adverse temperatures, pH, salinity, and finally, ability to be synthesized from renewable raw materials (Luna et al., 2012; Nalini and Parthasarathi, 2013; Souza et al., 2014b).

A major problem in biosurfactant production is the costs involved in the process. The carbon source is responsible by considerate part of biosurfactant production costs (Li et al., 2016). However, this may be significantly reduced by using alternative sources of nutrients, which are easily available and inexpensive. The use of industrial wastes as an energy source for biosurfactants production is an attractive alternative to decrease the production costs, making the process viable (Al-Bahry et al., 2013). Agro-industrial wastes with high content of carbohydrates, lipids and proteins are attractive to be used as substrate, they usually have the necessary nutrients for it (Nitschke and Pastore, 2002). Wastes such as glycerol (Souza et al., 2014a), petroleum sludge (Pirôllo, 2006; Cameotra and Singh, 2008; Ibrahim et al., 2013), sugarcane bagasse (Rakeshkumar et al., 2013) and fish waste (Aguiar et al., 2014) can be used such carbon source in fermentative process. In general, these raw materials can be used as distinct energy sources for different microorganisms with the capacity to produce biosurfactants. Several microorganisms are capable to biosurfactants production, among genus bacteria such as *Bacillus, Corynebacterium, Pseudomonas, Rhodococcus* (Souza et al., 2014b). Bacteria such as *Corynebacterium* spp. (Pinto et al., 2009; Deon et al., 2012; Decesaro et al., 2013). and *Corynebacterium aquaticum* (Pinto et al., 2009; Aguiar et al., 2014) are reported in the literature as producers of biosurfactants with low surface

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tension and with emulsifying capacity.

Petroleum-based compounds are highly pollutant when released to the environment and are considered largely responsible for the main causes of global pollution. A large number of these compounds are toxic and carcinogenic and may cause harm to human and animal health (Nalini and Parthasarathi, 2013). Among these compounds the solvents used for paint removal generally adhered to the surfaces which showed environment adverse impacts. For reduction of theses impacts is extremely important to search techniques with less aggressive toward pollutants (Young et al., 2015). According to Mulligan (2005) the biosurfactants are compounds that have several advantages compared with conventional surfactants which may be used in bioremediation processes (water and soil), enhanced oil recovery, solubilization of insoluble material, sequestering metals and removal and solubilization of paints.

Thus, the aim of this study was produce biosurfactants by *Corynebacterium aquaticum* and *Corynebacterium* spp. CCT 1968 using different carbon sources (fish waste, sugarcane bagasse, petroleum sludge and glycerol) and promotes biosurfactants application in paints removal from metallic surface.

2. Materials and methods

2.1. Material

The raw materials used as a carbon source for cultivation were sugarcane bagasse (SCB), fish waste (FW), crude glycerol (GL) and petroleum sludge from storage tanks (PS). The bagasse was obtained from farmers of Santo Antônio da Patrulha/RS, Brazil. The fish waste was provided by Pescal Company in the city of Rio Grande/RS, Brazil, the residue obtained was composed by heads, bones, skin, scales, muscles and viscera. Crude glycerol was provided by BS BIOS company, located in the city of Passo Fundo/RS, Brazil. The petroleum sludge remaining of storage tanks was donated by Oil Refinery Riograndense, located in Rio Grande/RS, Brazil.

2.2. Raw material preparation

The fish waste was reduced size using a mechanical removing device (HIGH TECH, HT/2500, Brazil). The fibers of bagasse were dried in an oven (Model Q-314 D 242 - Quimis) at 40 °C for 24 h and grounded (Mill Type Willye TE-650 - Tecnal) to reduce the particle size (100 mesh). The glycerol and sludge were homogenized manually.

2.3. Microorganisms

The microorganism *Corynebacterium aquaticum* was donated by Biochemical Engineering Laboratory (LEB) located at Federal University of Rio Grande (FURG) Brazil. This microorganism was isolated from washer trucks that carried hydrocarbons products. *Corynebacterium* spp. CCT 1968 was obtained by Tropical Culture Collection (CCT) donated by Foundation André Tosello, located in São Paulo/RS, Brazil.

The microorganisms were stored at 4 °C and propagated in Erlenmeyer flasks containing Agar Brain Heart Infusion (BHI) for cultivating of *Corynebacterium* spp. CCT 1968 and Agar Plate Count (PCA) for *Corynebacterium aquaticum*. Both were incubated at 37 °C for 48 h.

2.4. Cultivation medium

The aerobic cultivation was performed in erlenmeyer flasks (500 mL) at 30 °C and orbital shaking (Incubator Model TE-420 -Tecnal) 200 rpm for 48 h. The mineral medium was prepared as described by Yeh et al. (2005), consisting of NH_4NO_3 (50 mM), Na_2HPO_4 (3 mM), KH_2PO_4 (3 mM), $CaCl_2$ (7 μM), $MgSO_4.7H_2O$ (0.8 mM), EDTA sodium (4 μM), and $FeSO_4.7H_2O$ (2 mM). Carbon sources were used in concentrations of 3, 5 and 7%. After preparation of the culture medium, it was sterilized (AV Vertical Autoclave Model 30 - Phoenix) to eliminate some pre-existing contamination. The mineral medium was checked of surface tension by tensiometer (Kruss Processor Tensiometer K-9, Germany).

The addition of microorganisms was performed as described by Makkar and Cameotra (1998). The microorganisms presented in the surface of the agar in the erlenmeyer was removed and diluted in mineral medium until optical density between 0.8 and 0.9 (600 nm). The suspension was added in proportion of 2% (v/v). The microorganisms were added separately.

2.5. Evaluation of biosurfactants production

The biosurfactants production was evaluated at 0, 24 and 48 h of cultivation. The samples were taken from the reactor and centrifuged (Centrifuge Model MDW - 350 - Biosysten) for 30 min at 8667 g to remove cells and solid residues. Then, analysis were performed as described below.

2.5.1. Determination of surface tension

The surface tension (ST) of extract containing the biosurfactants was evaluated using a tensiometer (Kruss Processor Tensiometer K-9, Germany). This equipment uses the method of Du Nouy ring. This ring comes into contact with the liquid forming a film liquid/surface that is elongate to the breaking, measuring the maximum force used.

2.5.2. Emulsifying activity

The determination of oil emulsifying activity ($EA_{o/w}$) was analyzed according to the method described by Broderick and Cooney (1982). The extract containing the biosurfactants was homogenized with soybean oil using vortex and then, the samples rested for 24 h. The emulsifying activity was calculated by dividing the height of the emulsion layer by the total height of the mixture and multiplying by 100.

2.5.3. Character ionic of biosurfactant extract

The ionic character was determined as described by Meylheuc et al. (2001), using the technique of double diffusion in agar. This technique consists in the evaluation of two wells of the same distance created in low hardness agar (1% agar), one of them was added an ionic charge solution known and in the other, the biosurfactant. The anionic compound used was sodium dodecyl sulfate 20 mM and cationic solution used was barium chloride 50 mM. The appearance of the lines between the wells containing the biosurfactants and the ionic compound is able to suggest the ionic character of biosurfactants. The reading was made after 24 h of rest.

2.6. Biosurfactant application

The biosurfactants produced by *Corynebacterium aquaticum* using 3% of fish waste and 3% sugarcane bagasse were used to study the paint removal from metallic surface.

Synthetic enamel paint was used in the application process. The paint was composed of alkyd resin, organic and inorganic pigments, additives and solvent. The paint was insoluble in water and suitable for coating metal parts. The object used to support the paint was a metal plate (iron) 3 × 3 cm and 3 mm thickness. The metal plates were immersed in a container with paint, after it was removed from the container and suspended for remove the excess of paint. Thus, the plate containing the still wet layer of paint was placed into petri dish and covered with the respective solution for each treatment. The solutions used to cover the plates were previously selected. Four treatments were used, distilled water, solvent (turpentine solvent consisting of mixture of hydrocarbons), biosurfactant produced with fish waste and biosrufactant produced with sugarcane bagasse. The metal plates remained

for 15 days under orbital shaking (Incubator Model TE-420 - Tecnal) with a rotation of 50 rpm at room temperature. At 0, 1, 3, 5, 7, 10 and 15 days macroscopic analyzes were made of the appearance of metals subjected to each treatment.

3. Results and discussion

3.1. Surface tension and emulsifying activity of biosurfactants extracts

When alternative carbon sources are used, normally, the microorganisms use firstly the molecules of carbohydrates and fats (Nitschke and Costa, 2007). The FW has composed by 13.8% of fats and 0.5% of carbohydrates (data not showed). It is believed that the microorganisms were able to use the fats for biosurfactant production. In case of SBC as carbon source, the principal compound is the carbohydrates with 79% (data not showed). Thus, the microorganisms possibly used the carbohydrates for microorganism growth and biosurfactant production. According to the results obtained, the *Corynebacterium aquaticum* presented a good capacity in use both types of substrates.

Biosurfactants production using *Corynebacterium aquaticum* and FW as carbon source in 48 h of cultivation decreased the surface tension in approximately 25% (Fig. 1A). The concentrations of 3 and 5% of fish waste showed reductions in surface tension of 37.4 and 36.9 mN/m to 27.8 and 28.1 mN/m, respectively, in 48 h of fermentation. The surface tension reduction is an indicative that biosurfactants were produced into cultivation medium. The low initial surface tension may be assigned to the composition of FW. Residues are compounds by diverse structures and this can interfere in the surface tension.

The emulsifying activity values were between 65 and 95%. In the beginning of fermentation (time 0) the emulsifying activity of cultivation containing fish waste was high (Fig. 1A). According to He et al. (2013), proteins and peptides have functional properties, such as solubility, water holding capacity, viscosity, gelling ability, emulsifying properties, foam-forming ability and elasticity. The fish waste presents a high amount of proteins (14.4 ± 0.30 dates do not showed). Thus, the high emulsifying activity at the beginning of cultivation is assigned by the proteins from fish waste dispersed in the medium.

The experiment containing 3% of SCB (Fig. 1B) showed a reduction in the surface tension of approximately 21% regarding the initial surface tension. Rakeshkumar et al. (2013) used the microorganism *Klebsiella* sp. strain RJ-03 and several substrates, including sugarcane bagasse for the production of biosurfactants. These authors obtained surface tension of 47.1 mN/m in 72 h of cultivation, showing that is possible to obtain biosurfactant using SCB. However, in our studies, the surface tension obtained using the same substrate, but in other condition was 41.8 mN/m.

The use of SCB as carbon source for the development of biosurfactants showed high values of emulsifying activity (80.1%) for the experiment composed by 7% SCB in 48 h (Fig. 1B). The increasing values of emulsifying activity at 24 and 48 h are indicative of bioproduct production. Slivinski et al. (2012) produced biosurfactant by *Bacillus pumilus* UFPEDA448 using sugarcane bagasse and glycerol as substrate in concentration of 5 g/L. The authors obtained 52.5% of emulsifying activity in 72 h of cultivation. In comparison to their results, in the present study the higher emulsifying activity is an indicative of greater capacity of *Corynebacterium aquaticum* to use the nutrients contained in SCB.

Regarding the use of petroleum sludge, there was a low reduction (6%) of initial surface tension related to the final surface tension in 48 h (Fig. 2A). This behavior showed that this microorganism probably did not assimilate the compounds of petroleum sludge to biosurfactants production. Pirôllo (2006) used petroleum waste for biosurfactants production and observed that high biosurfactants production occurred when he used a higher petroleum concentration. The increase of substrate concentration generates stress to microorganism changing the metabolic route for biosurfactant production. The author also comments that the biosurfactant production using this substrate, in general, is attributed to a microorganisms isolated from contaminated

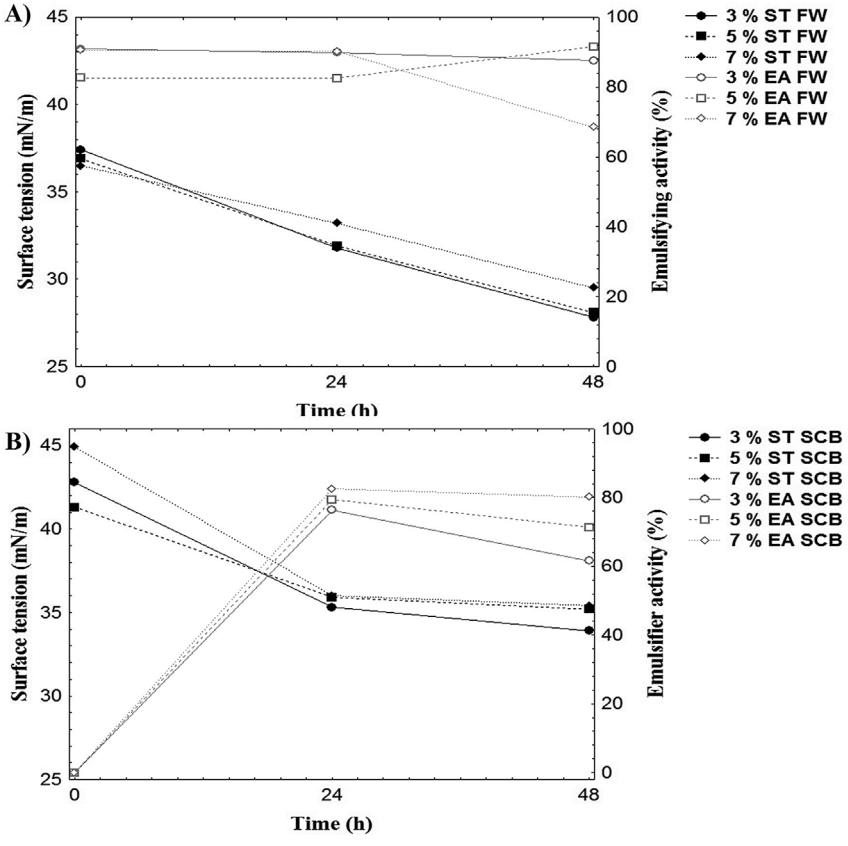

Fig. 1. Surface tension (ST) and emulsifying activity (EA) of biosurfactant produced using *Corynebacterium aquaticum* and (a) fish waste (FW) and (b) sugarcane bagasse (SCB).

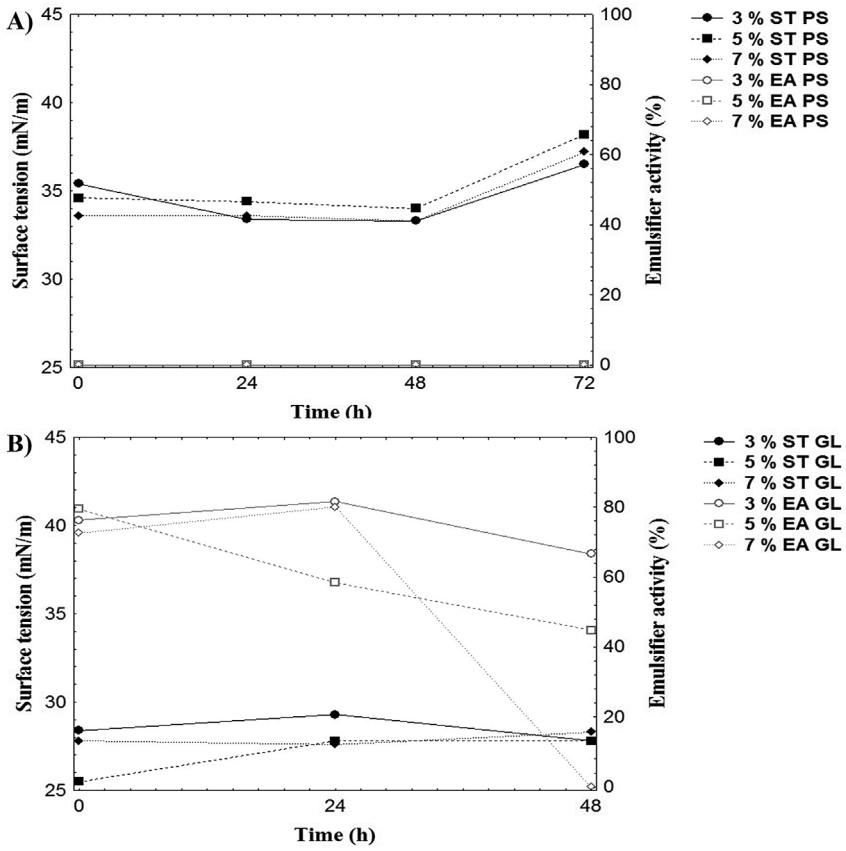

Fig. 2. Surface tension (ST) and emulsifying activity (EA) of biosurfactant produced using *Corynebacterium aquaticum* (a) petroleum sludge (PS) and (b) glycerol (GL).

hydrocarbons sites.

The use of glycerol as carbon source to obtain biosurfactants (Fig. 2B) demonstrate that this substrate may be was not used as carbon source for the biosurfactants production by this microorganism. The biosurfactant production, in generally, occurs when the surface tension reduces according to the fermentation process going on, but, in this case, it did not happen. Zouari et al. (2015) believed that very low initial surface tension in the beginning of cultivation with glycerol is because of the characteristics of glycerol structure, which is considered a surfactant. Silva et al. (2010) used the residual glycerol for biosurfactants production with *Pseudomonas aeruginosa* UCP0992. The results showed by these authors are an indicative that the biosurfactants production using glycerol is possible. Thus, biosurfactants production by *Corynebacterium aquaticum* using glycerol as carbon source were not efficient, in our study.

Fish waste used as carbon source to obtain biosurfactants using *Corynebacterium* spp. CCT 1968 (Table 1) showed a maximum decrease in surface tension at 48 h of cultivation. The use of 3% of the residue allowed to obtain the extract with the lower surface tension (28.5 mN/m). The high emulsifying activity showed the same behavior presented in the Fig. 1A that may be explained for protein concentration. The decrease in emulsifying activity throughout the process suggests that proteins promoted the initial emulsifying activity and then suffered action of microorganisms reducing their emulsifying properties.

The use of sugarcane bagasse as carbon source by the microorganism *Corynebacterium* spp. CCT 1968 showed a small decrease in the initial surface tension during the cultivation. The use of sugarcane bagasse was mention in the literature by several studies (Slivinski et al., 2012; Rakeshkumar et al., 2013; Sarubbo et al., 2015).

The cultivation carried out using SCB as carbon source obtained emulsifying activity of 88.3% at 48 h (Table 1). Although the surface tension measured for the biosurfactant produced from this substrate did not show large decrease, both analysis are not necessarily linked. A biosurfactant may have better emulsifying capacity than being a good surface tension reducing agent. This behavior is linked to the type of biosurfactant which is produced in the medium. According Christofi and Ivshina (2002), the biosurfactant can be segregated according to their molecular weight. Biosurfactants with low molecular weight have better performance in the reduction of surface and interfacial tensions, while the biosurfactants with high molecular weight act more effectively in emulsification processes.

The surface tension obtained in culture using petroleum sludge (Table 1) had a slightly change during cultivation, so it is not possible to evaluate the presence of biosurfactants. The same behavior was found for the cultivation carried out with glycerol. Through surface tension it was not possible to verify the biosurfactants production, due to the low variation of surface tension. The use of glycerol as carbon source for the cultivation containing *Corynebacterium* spp. CCT 1968 showed high emulsifying activity at time zero and then it has been declining during the cultivation. The high initial emulsifying activity is attributed to components usually present in crude glycerin, for example, soaps obtained from the biodiesel process. Additionally, glycerol can also be considered surfactant.

Although the cultivations were composed of mineral medium, it showed inert to the surface tension parameter, since the measurement of the surface tension of mineral medium value obtained 69 mN/m, value near to the surface tension of water that is around 72 mN/m. Thus, variations in the surface tensions at time zero, may be attributed only to the carbon sources presents in the medium.

According to Batista et al. (2006) a biosurfactant is considered effective when it has the ability to decrease surface tension to values below 40 mN/m and to stabilize emulsions at least 50% of the total volume for 24 h. The biosurfactants production by *Corynebacterium aquaticum* using fish waste and sugarcane bagasse as carbon source showed good results. Surface tensions obtained using fish waste as carbon source showed values below 30 mN/m in the better cases. For

Table 1
Surface tension and emulsifying activity obtained in the cultivation by *Corynebacterium* spp. CCT 1968.

Residue	C[a]	Surface tension[b] (mN/m)			Emulsifying activity[b] (%)		
		0 (h)	24 (h)	48 (h)	0 (h)	24 (h)	48 (h)
Fish waste	3%	$35.1^{a,A} \pm 0.1$	$28.9^{b,A} \pm 0.1$	$28.5^{b,B} \pm 0.1$	$69.2^{A} \pm 2.3$	0	0
	5%	$34.8^{a,B} \pm 0.0$	$32.8^{b,B} \pm 0.5$	$30.2^{c,A,B} \pm 1.7$	$69.5^{A} \pm 1.8$	0	0
	7%	$35.0^{a,A,B} \pm 0.2$	$34.4^{a,C} \pm 0.4$	$31.8^{b,A} \pm 0.3$	$70.4^{a,A} \pm 1.6$	$23.7^{b} \pm 2.1$	0
Sugarcane bagasse	3%	$40.4^{a,A} \pm 1.9$	$36.0^{b,C} \pm 1.4$	$43.0^{a,A,B} \pm 1.4$	0	$25.8^{b,C} \pm 1.2$	$24.6^{b,B} \pm 2.9$
	5%	$42.1^{a,A} \pm 2.1$	$39.9^{a,B} \pm 0,5$	$39.5^{a,B} \pm 1.2$	0	$30.2^{c,B} \pm 1.5$	$88.3^{bA} \pm 0.5$
	7%	$43.0^{a,A} \pm 1.1$	$42.9^{a,A} \pm 1.1$	$43.4^{a,A} \pm 1.5$	0	$34.1^{c,A} \pm 1.4$	$86.3^{b,A} \pm 0.1$
Petroleum sludge	3%	$38.1^{a,A} \pm 0.1$	$37.8^{a,A} \pm 0.9$	$38.4^{a,A} \pm 0.2$	0	0	0
	5%	$37.2^{a,B} \pm 0.5$	$37.2^{a,A} \pm 0,1$	$37.1^{a,B} \pm 0.4$	0	0	0
	7%	$38.4^{a,A} \pm 0.2$	$37.7^{b,A} \pm 0.2$	$37.6^{b,B} \pm 0.2$	0	0	0
Glycerol	3%	$30.0^{a,A} \pm 0.5$	$28.7^{b,B} \pm 0.1$	$29.3^{a,b,A} \pm 0.5$	0	0	0
	5%	$29.7^{a,A} \pm 0.7$	$29.4^{a,A} \pm 0.4$	$29.8^{a,A} \pm 0.0$	$16.7^{a,A} \pm 2.0$	$18.8^{a,A} \pm 3.0$	$20.2^{a} \pm 1.9$
	7%	$29.3^{a,A} \pm 0.1$	$29.5^{a,A} \pm 0.2$	$29.6^{a,A} \pm 0.2$	$41.2^{a,B} \pm 0,0$	$30.3^{b,B} \pm 1.5$	0

[a] C: concentration of waste used as carbon source.
[b] Mean ± standard deviation. In the same line for the answers obtained in the same analysis, means with different lowercase letters show difference significant at 5% probability by Tukey test. Means with different capital letters in the same column for the same residue show difference significant at 5% probability by Tukey test.

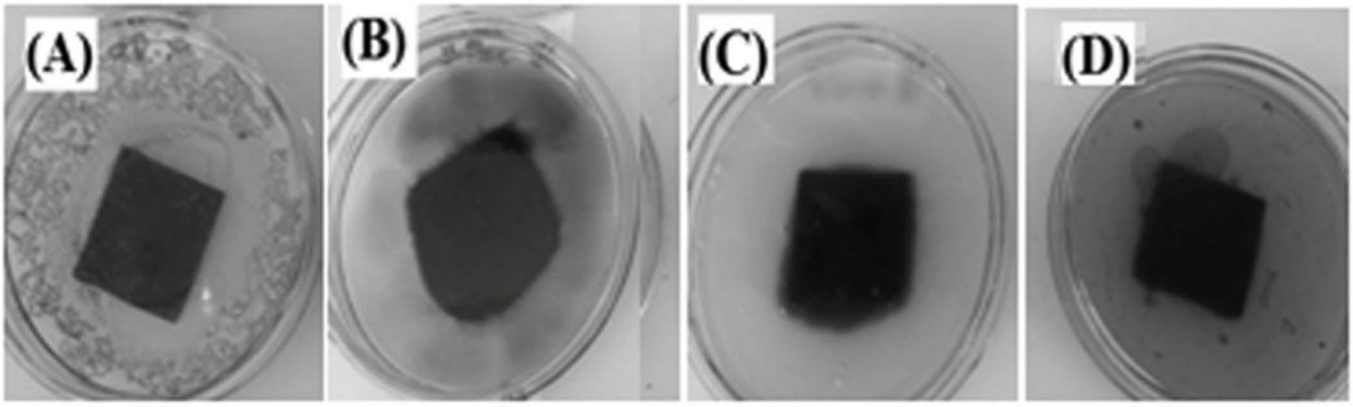

Fig. 3. Metal plates coated with paint and dipped in (a) water, (b) solvent, (c) biosurfactant of fish waste and (d) biosurfactant sugarcane bagasse at time zero.

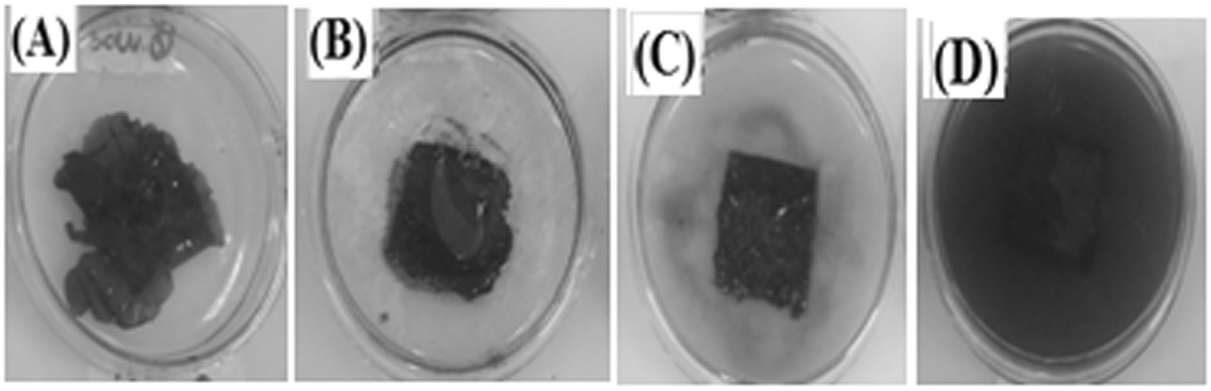

Fig. 4. Metal plates coated with paint and dipped in (a) water, (b) solvent, (c) biosurfactant of fish waste and (d) biosurfactant sugarcane bagasse at time 15 d.

the production of biosurfactants using the same microorganism and substituting the carbon source by sugarcane bagasse, surface tension showed values around 33 mN/m and highest values of emulsifying activity above 80%. The production of biosurfactants is directly linked to the microorganism producer, substrate used and process conditions.

The surfactin is a commercial biosurfactant. This bioproduct has the ability to reduce the surface tension of water from 72 to 28 mN/m (Barros et al., 2007). The reduction in surface tension results obtained by the biosurfactants produced from fish waste by both microorganisms indicate similar capacity to those biosurfactants.

The biosurfactants may be applied in several industries. The application is directly linked with biosurfactants properties. Biosurfactants with high emulsifying capacity may be used such as emulsifying and solubilizer agent for food products. In contrast, biosurfactants with high capacity in reduce the surfaces tensions may be used in solubilization process (reducing the area of contact) and bioremediation process. Thus, both biosurfactants that showed better results (biosurfactants produced by *Corynebacterium aquaticum* with 3% FW and 3% SCB) may be used in several industrial processes.

3.2. Ionic character of biosurfactant extracts

The ionic character of the bioproducts is an indicative for future applications. The determination of ionic character of biosurfactants is considered an important parameter because it directs the use of these compounds. Regardless the charge of biotensoactive, these can be used as emulsifying, surfactants or agent in bioremediation processes in environments contaminated with pollutants. However, only biosurfactants that have negative charges can be used as removing agents in soil remediation processes and waters contaminated with metals. The anionic charge of biosurfactants attracts the cationic ion of metal and reduces the metal availability in the environment. When relating biosurfactant with conventional surfactants, which may or may not have the ability to remove metals, biotensoactives are advantageous because, having low toxicity, are biodegradable and derived from renewable sources.

For biosurfactants extracts produced by *Corynebacterium aquaticum* the cultivations using fish waste and glycerol showed anionic character. In cultivations using petroleum sludge and sugarcane bagasse was not possible to observe the ionic character. Using *Corynebacterium* spp. CCT 1968 to produce biosurfactants from fish waste and sugarcane bagasse were verified anionic character for both of them. The other extracts (sludge and glycerol) did not show the halos making impossible to check the biosurfactants character.

Silva et al. (2010) performed the same experiment to determine the ionic character of the biosurfactant produced by *Pseudomonas aeruginosa* using glycerol as substrate and obtained confirmation of anionic character of biosurfactants. Meylheuc et al. (2001) determined the ionic character of the produced biosurfactants by *Pseudomonas fluorescens* 495 and found the diffusion of biosurfactant and halos formation near the barium chloride solutions and cetyl trimethyl ammonium bromide (CTAB), both solutions with positive character highlighting the anionic character of bioproduct.

3.3. Application of biosurfactants

Fig. 3 shows the moment when the plates covered by paint were immersed in the respective solutions. The metals with fresh paint were immersed in the solvents before they were completely dry. The analysis were carried out with fresh paint trying to simulate problems founded with the containers that are used to carrier fresh paint in ships, those containers need to be cleaned to come back to the industries and then are necessary to use toxic solvents, as xylene and toluene, to wash them. Using biosurfactants to wash those containers could be a good solution to have less aggressive compounds to be treated, and also it is more safely for the handlers and environment.

The structure of the biosurfactant is extremely important in removal paint process. The amphipathic molecule of biosurfactants can connect to the material insoluble in water and at the same time increase water dispersion and solubilization of different compounds (Silva et al., 2014). In the moment of application, it was observed that the biosurfactant was removing the paint from the metallic surface and solubilizing the paint in the aqueous extract. The principal solvent of biosurfactants in the fermentation was mineral medium. This medium is composite with salts and water, thus the medium mineral did not present influence in solubilization and removal of paint. The action of paint removal, in this case, is assigned to the biosurfactants.

Observing the Figs. 3 and 4 the metallic plates have several differences. It can be seen, at the zero time, that the plates immersed in water showed paint remained in the water surface in the undiluted form (Fig. 3A), while the plates immersed in solvent (Fig. 3B) showed a small amount of paint diluted in the solvent. The plates immersed in the medium containing the biosurfactants produced from fish waste (Fig. 3C), initially, did not interact with the bioproduct, and the plates containing the biosurfactants produced from sugarcane bagasse (Fig. 3D), showed a portion solubilized in the medium.

In the assay using the biosurfactants produced from FW (15 d) is possible to observe diluted paint in the medium (Fig. 4C). This behavior showed us that bioproduct was able to remove the paint from metallic surface. The assay using the biosurfactants produced from bagasse, compared with the zero time is darker, although it did not remove totally the paint. Part of the paint removed was solubilized in the medium (Fig. 4D).

The plate that was immerse in water, after 15 d, did not show any paint removal, the stain presented in the Fig. 4A is in the petri dish bottom and not solubilized paint. The plate containing appropriate solvent reached the solubilization of the paint in the medium and after this process the paint adhered to the metal surface again, but it was easily removed. The use of biosurfactants produce by fish waste did not promote the paint solubilization, however the paint layer showed thinner than the metal surface treated with water. The biosurfactants produced from sugarcane bagasse was the most promising in the use as paint agent removal and in the solubilization process. Once, this could remove most of the paint adhered to the metallic surface and solubilized in medium containing biosurfactant extract. This behavior can be attributed to the high activity emulsifying showed for this bio-compound (80.1%).

According to Mulligan (2005) the biosurfactants have several potential uses in industry. Among these possibilities for application we can find solubilize paints. However, in the literature there is no information about the use of biosurfactants as surface paint removing agent. It is known that biosurfactants are molecules able to solubilize a variety of hydrophobic components from different material, as vegetable oils or products from petroleum.

4. Conclusion

The microorganisms *Corynebacterium aquaticum* and *Corynebacterium* spp. CTT 1968 showed efficient production of biosurfactants. Among the industrial wastes used, the fish waste and sugarcane bagasse were more efficient as carbon source for the bioproducts produced. The biosurfactants produced with these carbon sources showed surface tension reduction to values below 40 mN/m. They were also able to maintain the emulsifying activity above 50%. The petroleum sludge and glycerol were not effective in biosurfactant production. Biosurfactant extracts studied were considered with anionic character. The biosurfactants produced from sugarcane bagasse showed promising in the solubilization and paint removal.

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