

Full Length Research Paper

Adsorption of copper, zinc and lead on biosurfactant produced from cassava wastewater

Larissa Kummer^{1*}, Natássia Jersak Cosmann², Glaucia Maria Pastore³, Ana Paula Resende Simiqueli³, Vander de Freitas Melo⁴ and Simone Damasceno Gomes²

¹Department of Chemistry and Biology, Federal Technological University of Paraná (UTFPR). Rua Deputado Heitor Alencar Furtado, 5000 - 81280-340 Curitiba-PR, Brazil.

²Center of Exact and Technological Sciences, Western Paraná State University (UNIOESTE/CASCADEL/CCET/PGEAGRI), Rua Universitária, 2069 - 85819-110 Cascavel, PR, Brazil.

³Department of Food Science, Faculty of Food Engineering, State University of Campinas, Rua Monteiro Lobato, 13083-862 Campinas, SP, Brazil.

⁴Department of Soil Science and Agricultural Engineering, Federal University of Paraná (UFPR), Rua dos Funcionários, 1540 - 80035-050 Curitiba, PR, Brazil.

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The remediation of soils contaminated by hydrophobic compounds, especially petroleum derivatives, using surfactants has been fairly studied. The use of these compounds in heavy metal contaminated areas is incipient and their mechanism of action has not been totally elucidated yet. The biosurfactants are compounds with surfactant characteristics produced by living organisms and have several environmental advantages, like greater biodegradability and production from renewable sources. The aim of this study was to evaluate the adsorption potential of the metals copper, zinc and lead onto the biosurfactant structure and to relate it to the Langmuir and Freundlich physicochemical models of adsorption. The biosurfactant produced presented an emulsification index (EI) around 60%, which was stable even after 72 h of emulsion formation. The analysis of FTIR and HPLC confirmed that the biosurfactant produced is composed mainly of surfactin, found in concentration of 28%. The biosurfactant was capable of adsorbing the metals in its structure. In the adsorption process of Pb^{2+} , the biosurfactant was considered as an interesting adsorbent, but this behavior did not adjust satisfactorily to Langmuir and Freundlich models. Thus, the biosurfactant has the potential to be utilized in remediation processes of soils and treatment of effluents contaminated by heavy metals.

Key words: *Bacillus subtilis*, trace elements, heavy metals, remediation of contaminated areas, agro industrial residue, surfactin.

INTRODUCTION

The surfactants are an important class of chemicals widely utilized in several industrial sectors, as

pharmaceutical, cosmetic, fine chemical, food and environmental applications, and are usually synthesized

*Corresponding author. E-mail: lkummer@utfpr.edu.br. Tel: (55 41) 3279-4575.

from petroleum derivatives (Silva et al., 2010). However, the growth of environmental concern between the consumers allied with new legislations of environmental control, lead to the pursuit of natural surfactants as an alternative to the available products. Biosurfactants are bacteria, fungi and yeast metabolic byproducts that show surfactant properties (Barros et al., 2007; Das et al., 2009; Marin et al., 2015; Cortés-Camargo et al., 2016). The application of surfactants produced biotechnologically is restricted to certain areas, since the production process is still not economically competitive as compared to surfactants derived from petroleum (Henkel et al., 2012). Biosurfactant production in industrial scale is usually limited by the high cost of culture media, associated with inefficient downstream methods and to relatively low incomes of the product (Henkel et al., 2012). The use of agroindustrial wastes as substrates for microbial production has been suggested as an alternative to make the process commercially feasible (Colla and Costa, 2003; Barros et al., 2007; Bezerra et al., 2012; Henkel et al., 2012) and enables, as well, the utilization of million tons of environmentally dangerous residues in biotechnological processes (Cortés-Camargo et al., 2016).

Different industrial wastes were studied for biotechnological purposes, such as: cooking oil residues (Haba et al., 2000), effluents of olive oil production (Mercade et al., 1993), molasses and whey (Joshi et al., 2008), okara - solid residue from the production of soy milk (Slivinski et al., 2012), vine-trimming wastes (Cortés-Camargo et al., 2016) and liquor from sisal (*Agave sisalana*) pulp hydrolysis (Marin et al., 2015).

World cassava production is around 263 million tons (Mt) per year with Africa accounting for about 57% of the supply in 2012. Asia is second with 30.7%, while Latin America and the Caribbean are third with 12.2%. Nigeria is the largest producer of cassava in the world with more than 54 Mt at the end of 2012 and The Democratic Republic of Congo is the second largest producer of cassava in Africa after Nigeria in 2012 (FAOSTAT, 2014). A positive characteristic is that cassava crop is very resilient and can be cultivated in a wide variety of ecological zones, making it ideal for poor farmers to cultivate (Okudoh et al., 2014).

The industrial processing of the cassava generates residues that require different treatments for their disposition that are conditioned to the technological and economic level of each company. The liquid residue, cassava wastewater, is generated during the process of shredded mass pressing for flour production or during cassava starch extraction and purification (Suman et al., 2011). The high concentration of remaining carbohydrates and several important micronutrients can cause a serious problem of environmental pollution if the effluent is not properly treated before disposition (Damasceno et al., 2003). On the other hand, this is also an interesting characteristic in a culture medium, since there might be no need for supplementation to biosurfactant production

(Barros et al., 2007; Bezerra et al., 2012).

The application of surfactants in order to remediate soils contaminated with hydrocarbons and/or heavy metals has been widely studied and the biosurfactants are an attractive option because of their biodegradability and lower toxicity (Song et al., 2008; Wan et al., 2011). The efficiency of these compounds for the efficient removal of metals depends on several physicochemical mechanisms of interaction and environmental conditions that have still not been totally elucidated (Franzetti et al., 2009; Singh and Turner, 2009). Among the basic factors involved, the types of surfactant, soil and contaminant, as well the complex interaction between them may be cited (Juwarkar et al., 2007; Asçi et al., 2010; Torres et al., 2012). According to Ramamurthy et al. (2008), anionic surfactants have the largest effects on metals mobilization. Burrows et al. (2010) reported the surfactant acidity as a determining factor for its larger or smaller interaction with the metals, which is stronger for slightly acidic carboxylates than for sulphates or sulphonates. Although these interactions should be due in great part to electrostatic effects, they are also, in some cases, followed by an increase in entropy as a result of the release of water from hydrated cations.

Stalikas (2002) discussed that the interaction of metal species with surfactants occurs through electrostatic interactions with the surfactant polar head, or by hydrophobic interactions of the metal chelates with the micelles hydrophobic chain, which could be a strategy to, for example, promote the removal of metallic ions from an effluent (Costa, 2011).

Rhamnolipid-type biosurfactant interaction with metals was reported by Wang and Mulligan (2009), whose results suggested that the interaction of biosurfactant with soils and/or residues containing heavy metals, may occur due to several factors, such as interfacial tension reduction and solubilization of metals by the formation of micelles or complexes. Das et al. (2009) tested a biosurfactant produced by *Bacillus circulans* in different critical micellar concentrations (CMC) and concluded that as the concentration of the surfactant increases, the metal removal (Cd and Pb) of the environment (liquid effluent) also increased, reaching a complete removal with a concentration corresponding to 5xCMC.

Thus, this study aimed to evaluate the adsorption capacity of heavy metals (Cu^{2+} , Zn^{2+} and Pb^{2+}) into the biosurfactant obtained using cassava wastewater, liquid waste from cassava flour production, in order to assess its use as a possible alternative in decontamination of effluents and soils contaminated with metals.

MATERIALS AND METHODS

Biosurfactant production

The biosurfactant was produced by *Bacillus subtilis* LB5a isolates pertaining to the culture collection of the Bioflavors Laboratory of

DCA/FEA/Unicamp (Brazil). The liquid wastewater from the cassava flour, the cassava water, was previously treated by heating until boiling point, then chilled and centrifuged to be used as culture medium. The fermentation process was carried out in a pilot fermenter (Pilot New Brunswick 8000 MP 80), at 30°C, 4 vvm (air volume per volume of culture medium per minute) and agitation of 150 rpm (Barros et al., 2008a).

The foam produced during the fermentation process was continuously collected and collapsed (Barros et al., 2007). For biosurfactant extraction, the collapsed foam was centrifuged at 12.700 G for 10 min to get cell-free solution, then had its pH adjusted to 2.0 using 6N HCl and was kept at 4°C overnight for decantation. After, the liquid was centrifuged at 12.700 G per 20 min, the precipitate was collected, neutralized with 3 M NaOH, and then dried at 50°C 50²+C (Makkar and Cameotra, 1997, 1999; Barros et al., 2007). This material, named raw biosurfactant, was then crushed in mortar and stored for later analysis.

Emulsification index

Screw cap test tubes containing 6 mL of hydrocarbon and 4 mL of a 48 mg.L⁻¹ solution of raw biosurfactant were vortexed for 2 min and then left to rest at room temperature. The hydrophobic compounds utilized were sunflower oil, soybean oil and gasoline. The emulsification index was determined after 24, 48 and 72 h (EI_{24h}, EI_{48h} and EI_{72h}, respectively) by the difference between the height of the emulsion layer (EL) and the mixture's total height (TH), as shown in Equation 1 (Cooper and Goldenberg, 1987):

$$EI (\%) = (EL/TH) \times 100 \quad 1$$

Biosurfactant characterization by infrared spectroscopy

The biosurfactant obtained was characterized by Fourier transform mid-infrared spectroscopy (FT-MIR), using the Shimadzu IR Prestige 21 infrared spectrophotometer with detector DTGS. A quantity of 2 to 5 mg of the samples were diluted in 100 mg of KBr and analyzed by the transmission technique.

The organic attributions for the observed spectroscopic bands were done by the comparative method with the data available in Silverstein and Webster (2007) and other papers in the area to confirm the associations, which were presented during the discussion of the obtained results.

Biosurfactant characterization by high performance liquid chromatography

The biosurfactant was analyzed by high performance liquid chromatography (HPLC) utilizing a Shimadzu liquid chromatographer, model LC 20 AT, with a Kromasil C18 column. The mobile phase used was acetonitrile and trifluoroacetic acid (3,8 mmol.L⁻¹) in ratio of 80:20.

To confirm the surfactant type, a co-chromatography with surfactin standard from Sigma- Aldrich was performed. Both the biosurfactant and the standard sample were diluted in acetonitrile: methanol (1:1, v:v) (2 mg.mL⁻¹) and 20 µL were used for the injection. This injection methodology was based on Slivinski et al. (2012).

Adsorption Isotherms for the ions Cu²⁺, Zn²⁺ and Pb²⁺

A volume of 20 mL of several solutions containing metallic ions in different concentrations were brought into contact with 50 mg of

adsorbent (biosurfactant) at 25°C, agitation of 200 rpm and pH ~ 6.3. The pH of the metallic solutions was adjusted using NaOH (0.1 mol.L⁻¹) and was not corrected during the experiments of adsorption. In order to prevent an increase in competition of the different species for the adsorption spots, a pH buffer was not used. The metallic solutions were prepared from commercial analytical standards of 1,000 mg.L⁻¹ and then diluted to concentrations between 50 and 750 mg.L⁻¹ for Zn²⁺ and from 20 to 200 mg.L⁻¹ for both Cu²⁺ and Pb²⁺. All the tests were performed in triplicate.

After 24 h of shaking to reach the balance, the samples were centrifuged at 3,500 G for 10 min and then filtered through qualitative filter paper. The metal concentration in the supernatants was measured by atomic adsorption spectroscopy (AAS) in a Shimadzu spectrophotometer model AA 6030. Isotherms models of Langmuir and Freündlich were applied to the obtained data.

The quantity of adsorbed metal (q) was the difference between the initial concentration of metal in each solution and the concentration in the supernatant after contact with the adsorbent (C_e). The data collected experimentally were adjusted to the models of Langmuir and Freündlich whose equations are presented below. The isotherm of Langmuir is given by the Equation 2 where: q_e = quantity of adsorbed metal per mass adsorbent unity (mg.kg⁻¹); K_L = Langmuir constant related to the connection energy of the metal to the adsorbent (L.mg⁻¹); C_e = metal concentration in the balance solution (mg.L⁻¹).

$$\frac{C_e}{q_e} = \frac{1}{K_L b} + \frac{C_e}{b} \quad 2$$

The Freündlich equation is presented in Equation 3, where q_e = amount of metals adsorbed per unit mass of the adsorbent (mg.kg⁻¹); K_F = Freündlich adsorption constant (L.mg⁻¹); n = Freündlich constant related to surface heterogeneity (dimensionless); C_e = metal concentration in the balance solution (mg.L⁻¹)

$$q_e = K_F C_e^{\frac{1}{n}} \quad 3$$

RESULTS AND DISCUSSION

Emulsification index (EI)

The EI_{24h} for the biosurfactant solution with the three types of oil evaluated, sunflower oil, soybean oil and gasoline, were about 60% and it was observed that the emulsions formed were stable for at least 72 h (Table 1). Bezerra et al. (2012) who produced ramnolipid from cassava water with *Pseudomonas aeruginosa* obtained the emulsification index of the biosurfactant solution with kerosene ranging from 50 to 68% for the first 24 h. A lot of them are maintained stable for 72 h or more. Batista et al. (2006) that worked with surfactin, got values of 50% of the EI for the commercial kerosene. Barros et al. (2008b) analyzed the surfactin obtained from *B. subtilis* Lb5a and produced under the same conditions as described in the present study with 15 different types of hydrophobic compounds. The emulsions with sunflower

Table 1. Emulsification index (EI) of different emulsions of hydrocarbons with the biosurfactant produced by *Bacillus subtilis* LB5a.

Hydrophobic compound	EI (%)		
	24h	48h	72h
Soybean oil	59.4	59.4	59.4
Sunflower oil	59.7	59.7	59.7
Gasoline	60.3	60.3	60.3

oil, soybean oil and gasoline were stable for at least 72 h and values of EI_{24h} were close to 70%. This difference is probably due to the level of purification of the surfactin used, as the biosurfactant used by Barros et al. (2008b) had gone through an additional process of extraction with solvents. Besides, these same authors verified the relation between the sizes of the fatty acids carbonic chains in the tested oils and the EI_{24h} of the emulsions with surfactin and could not identify any relation between the type and the size of the chains. The stability of the emulsions found in the present study and also reported in other papers might indicate that, once the metal is adsorbed into the biosurfactant, the possibility of desorption of the metal to the environment is reduced.

Biosurfactant characterization

The results have confirmed that the biosurfactant obtained in this work is really surfactin, once the bands are very similar to the standard of surfactin (Sigma-Aldrich) (Figure 1). The Fourier transform infrared spectroscopy (FTIR) is a methodology that detects similarities or differences in the chemical structures of compounds (Franca et al., 2010). According to Silverstein and Webster (2007), functional groups and structures can be discriminated by their molecular vibrations. This technique can be used for the identification of the biosurfactants because most of them have one radical carbonyl, ester binding or carboxylic acid, which absorb energy in the infrared region of the electromagnetic spectrum (Gartshore et al., 2000).

The absorption spectrum in the infrared zone of the biosurfactant was compared with the spectrum of surfactin standard from Sigma Aldrich (Figure 1), as well as to the data described by Teixeira et al. (2009) and Oliveira et al. (2013) studies that obtained surfactin using different carbon sources. Oliveira et al. (2013) used clarified cashew juice and Teixeira et al. (2009), artificial mineral nutrient broth.

It is possible to observe an absorption in 3.282 cm^{-1} , characteristic of axial strain of the connection N-H present in peptides; absorption in 2.952 cm^{-1} corresponds to the asymmetric stretching of the connection C-H of grouping CH_3 (fatty acids); band in 2.922 cm^{-1}

correspondent to the asymmetric stretching of the connection C-H of grouping CH_2 ; band of axial strain C=O in 1.720 cm^{-1} of carboxylic acid. This band was observed in all works used as comparison and also in all of them, this strain is discrete (Teixeira et al., 2009; Oliveira et al., 2013). There are two absorptions, one in 1.647 cm^{-1} , that feature the amide I group (C=O; C=N), and other in 1.535 cm^{-1} that feature the amide II. Oliveira et al. (2013) also obtained a very similar spectrum, assigning such bands likewise to the amide groups. In the zone of 1.230 to 1.200 cm^{-1} , the vibration C=O in aromatic ring or CH_2 strain in alkyl carboxyl is possible. The strain in 1.056 cm^{-1} can represent the C-O stretch of polysaccharides.

The area obtained from the integration of the 5 peaks of the homologous by HPLC was applied in the regression equation calculated through the calibration curve constructed using different concentrations of surfactin standard from Sigma-Aldrich ($R^2 = 0.9904$) to estimate surfactin concentration in the raw biosurfactant. The elution profile in HPLC of the homologous lipopeptide produced presented retention times equivalent to the ones obtained for the standard surfactin. Doing the comparison of the chromatogram obtained for the biosurfactant produced with chromatogram of the standard Sigma-Aldrich sample, as well the results of FTIR and the literature results, it is possible to identify the surfactant produced as surfactin.

Five peaks have been identified: at 6, 14, 18, 25 and 27 min. The integration of the 5 peak areas showed that the concentration of surfactin in the raw biosurfactant was 28%. Since after acid precipitation the biosurfactant did not suffer any other downstream process, a low level of purity was expected. Oliveira et al. (2013) evaluated the biosurfactant production by *B. subtilis* using cashew juice and obtained 11.17% of surfactin in the semi-purified biosurfactant. Franca et al. (2010) produced biosurfactant from *Pseudomonas aeruginosa*. EQ 109 from glycerol and obtained low production yields, and after the purification process, the aforementioned authors obtained about 6.57% of rhamnolipid in biosurfactant.

The choice to avoid further steps of purification was made considering the intended use of this biosurfactant. It was reported that the extraction by solvents, and resulting increase in costs and generation of chemical residues, was not necessary for the process of remediation of soils or effluents.

Adsorption of metals onto the biosurfactant

In Figure 2, there are experimental data, as well as the obtained isotherms by the models of Langmuir and Freundlich for adsorption of Zn, Cu^{2+} and Pb^{2+} by the biosurfactant. Resulting parameters of the isotherms are listed in Table 2.

The high determination coefficients (R^2), obtained from

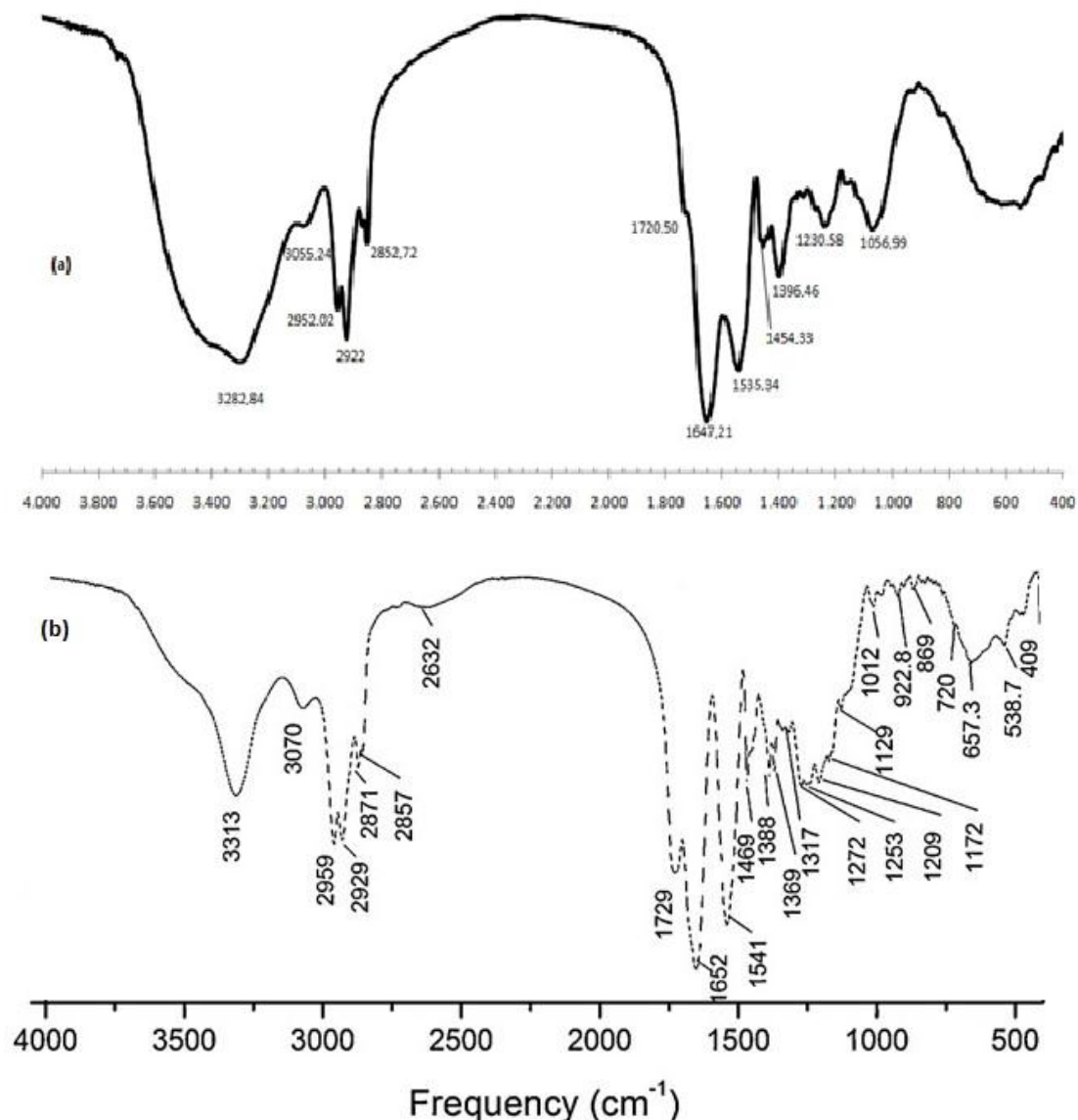


Figure 1. Infrared spectrum of the biosurfactant obtained by the bioprocess (a) and surfactin standard Sigma-Aldrich (b).

Table 2. Langmuir and Freundlich parameters for adsorption of Zn^{2+} , Cu^{2+} and Pb^{2+} into the biosurfactant.

Parameter	Langmuir			Freundlich		
	$K_L (L mg^{-1})$	$B (mg g^{-1})$	R^2	$K_F (L mg^{-1})$	$1/n$	R^2
Zinc	0.25	5.00	0.9981	100.79	1.21	0.8710
Copper	0.11	10.00	0.9879	303.53	1.04	0.8230
Lead	0.05	3.33	0.5355	32.02	2.08	0.8125

K_L - Langmuir isotherm constant; b - maximum monolayer coverage capacity; R^2 - coefficient of determination; K_F - Freundlich isotherm constant.

the linearized equations of Langmuir and Freundlich (Table 2), indicate that both adsorption models are

significantly able to demonstrate the adsorption of Zn^{2+} and Cu^{2+} into the studied biosurfactant.

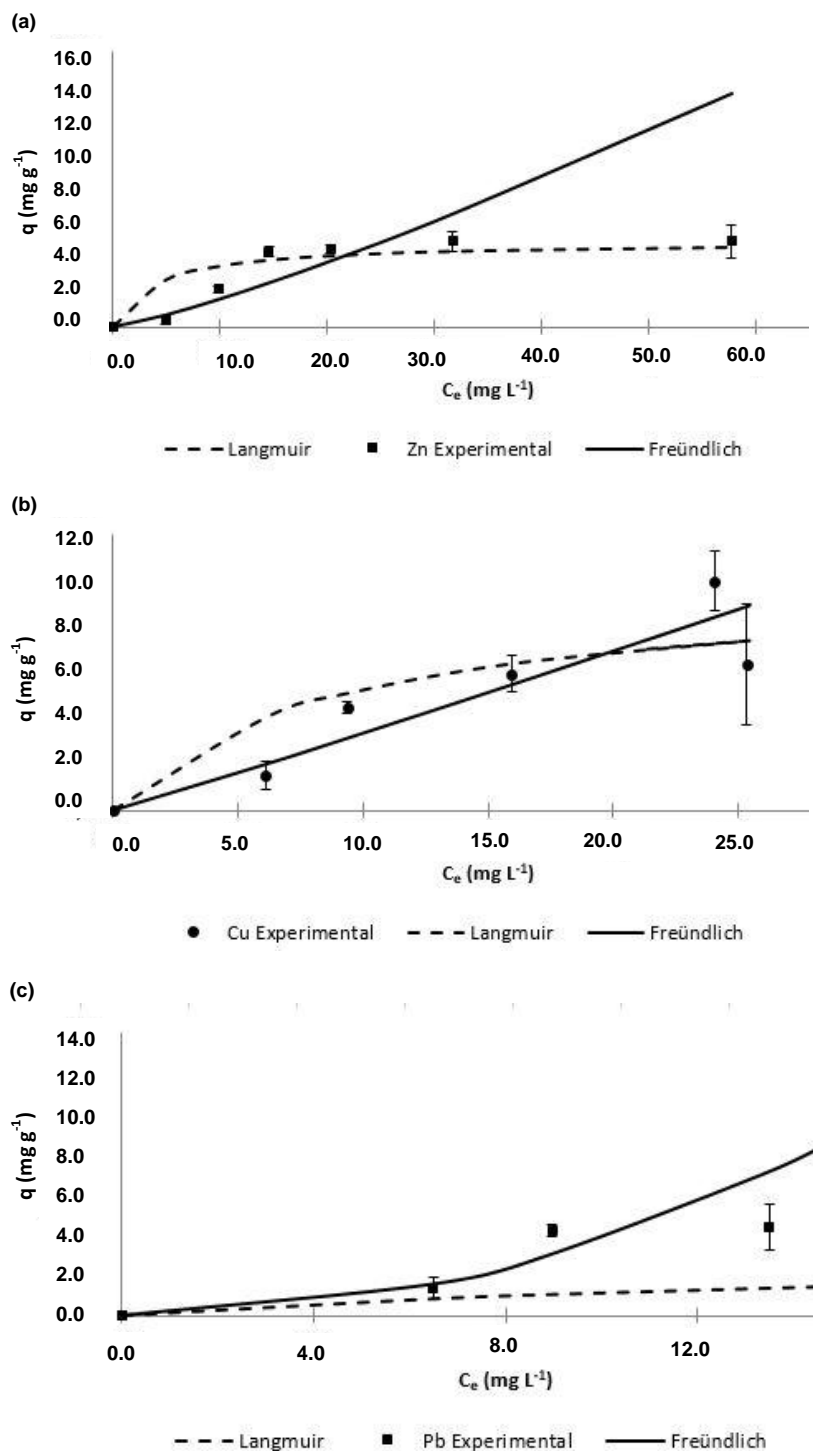


Figure 2. Isotherms obtained from the adsorption data obtained experimentally for Zn²⁺ (a) Cu²⁺ (b) and Pb²⁺ (c) in the equilibrium solution.

Langmuir model represented better the behavior of adsorption of Zn²⁺ into the biosurfactant, and Freundlich model overestimated the experimental values (Figure 2).

Based on the b values obtained by the Langmuir

isotherm, that estimates the maximum capacity of adsorption (MCA), it is possible to affirm that the biosurfactant has high affinity for the studied metals. The value for Cu²⁺ was the highest, 10 mg.g⁻¹, followed by Zn²⁺ (5 mg.g⁻¹). For Pb²⁺, the curves showed that the

system did not adjust well to the Langmuir model in the studied concentration range, which makes the MCA value not valid. Freundlich model was the most appropriate to describe the adsorption of this element.

The experimental values were important to confirm that the biosurfactant produced from cassava wastewater have shown high capacity to adsorb metals, independent whether an adjustment to the evaluated physicochemical models has been observed. According to Zeraik and Nitschke (2010) and Seydlová and Svobodová (2008), the surfactin is characterized as an anionic biosurfactant is a result of the negative charges in its polar group, which is due to the presence of functional groups as amide and carboxylic acids.

Therefore, this capacity to adsorb metals on the cation forms occurs because of the anionic nature of the surfactin, which allows the interaction between the negatives charges of the biosurfactant and the positives charges of the metallic ions. According to Piron and Domard (1997), who studied the uranium adsorption into different types of chitosans, the adsorption capacity increases with the average degree of acetylation (DA). The DA measures the percentage of amino groups available in the chitosan chain and is one of the main parameters that affect the adsorption properties.

Colla et al. (2012), in a study that compared the Cd removal in liquid containing biosurfactant and different species of filamentous fungi, concluded that in those liquids containing biosurfactant, the metal removal was better. The association between *Aspergillus* fungi and biosurfactant promoted 100% removal of the Cd existence in the solution. The authors concluded that the presence of biosurfactants in liquids improves the metal removal efficiency.

The difference between the adsorption potential for the three metals studied is due to the properties of each one of them. According to Vieira et al. (2011), concentration, pH and ionic strength of the solution, beyond the effect of competitors ions, widely influences the adsorption properties, besides contributing to the protonation of the amino groups from the biopolymers. Thus, the knowledge of the behavior of these metallic species in solution, may contribute to the understanding of the adsorption mechanism (ionic change, electrostatic action, chelation, etc).

According to Miller (1995), the biosurfactant presents potential to increase the efficiency of metal removal in soils because they facilitate sorption processes, mass transferences and resistance to transport in aqueous phase. The addition of biosurfactants may promote the desorption of heavy metals of solid phases through the complexation of free forms of metals in solution or by the accumulation of the biosurfactants in the liquid-solid interface, caused by the reduction of interfacial activities, which promotes a direct contact between the biosurfactants and the metals. Moreover, Valdman et al. (2005) demonstrated that an exopolysaccharide

extracted from the microorganism *Serratia* sp., with biosurfactant activities, presented a high efficiency in the Cd removal from liquids. In these experiments 50 mg.L⁻¹ of Cd²⁺ and 0.1 g.L⁻¹ of the previously purified biosurfactant were used, achieving a Cd removal of 170 mg.g⁻¹ of biosurfactant.

Conclusions

The biosurfactant produced presents emulsifier properties and stability, and the emulsification index was about 60% even after 72 h. The biosurfactant produced consists fundamentally of surfactin with 28% of purity.

The biosurfactant produced from cassava wastewater has shown capability to adsorb Cu²⁺, Zn²⁺ and Pb²⁺ into its structure. The maximum capacity of adsorption of Cu²⁺ and Zn²⁺ was estimated at 10 and 5 mg.g⁻¹, respectively.

In the adsorption process of Pb²⁺, the biosurfactant presented as a good adsorbent, however the satisfactory adjustment has not been observed for the Langmuir model. The Freundlich model presented better adjustment.

Further studies are recommended to verify the behavior of the biosurfactant in liquid effluents and soils containing heavy metals.

Conflicts of interest

The authors declare that there is no conflict of interest.

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