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Produção de surfactina utilizando extrato aquoso da casca de papaya como substrato e sua aplicação para adsorção de ferro

Surfactin production using papaya peel aqueous extract as substrate and its application for iron adsorption

Producción de surfactina utilizando extracto acuoso de la cáscara de papaya como sustrato y su aplicación para la adsorción de hierro

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Resumo

A presença de metais em efluentes industriais tornou-se um grande problema ambiental, uma vez que esses resíduos são frequentemente descartados em lagos ou rios. Com a intenção de recuperar áreas contaminadas, a remediação por lavagem com biossurfactantes aparece como uma alternativa que apresenta baixa toxicidade para o meio ambiente. O presente trabalho objetivou avaliar a eficiência na remoção do ferro em um efluente sintético, utilizando um biossurfactante produzido em um biorreator (37°C, 200 rpm, 0.5 vvm), a partir do extrato aquoso da casca de mamão e da cepa *Bacillus subtilis* UFPEDA 86. Os ensaios fermentativos mostraram que esse *Bacillus* é um bom produtor de biossurfactante e que o extrato da casca de mamão é um substrato viável para a produção de biossurfactante por esta cepa. Dentre os resultados encontrados, em 24 horas de cultivo, obteve-se a maior concentração de biomassa e produto, de 2,17 ± 0,04 $g.L^{-1}$ e 2,88 ± 0,01 $g.L^{-1}$, respectivamente. O biossurfactante produzido apresentou Concentração Micelar Crítica (CMC) de 20 $mg.L^{-1}$. Ensaios em batelada foram utilizados para obtenção dos dados de remoção, nos quais uma série de soluções em diferentes concentrações de íons de ferro foram expostas a diferentes quantidades de biossurfactante, bruto e purificado, a uma temperatura de 25 ° C, sob agitação (200 rpm) e pH ~ 6,3. Foi realizado um planejamento experimental multivariado, na presença de biossurfactante bruto e purificado, onde os resultados mostraram que as interações entre as variáveis independentes (concentração dos íons de ferro, concentração de biossurfactante e o tempo de tratamento) foram significantes para ambos. As porcentagens de remoção do ferro variaram entre 47,2 e 95,82%, na presença do bissurfactante bruto e de 37,01 a 91,94% na presença do biossurfactante purificado. O modelo de adsorção de Langmuir foi o melhor ajustado, sendo a capacidade máxima de adsorção estimada em 10 $mg.g^{-1}$.

Palavras-chave: *Bacillus subtilis*; Biossurfactante; Adsorção; Ferro.

Abstract

The presence of metals in industrial effluents has become a major environmental problem since these residues are often disposed of in lakes or rivers. Aiming to recover contaminated areas the remediation by washing using biosurfactants appears as an alternative technique that features low toxicity to the environment. This paper aims to evaluate the efficiency in iron removal within a synthetic effluent, utilizing a biosurfactant. This was produced in a bioreactor (37°C, 200 rpm, 0.5 vvm) derived from a papaya peel aqueous extract and the *Bacillus subtilis* UFPEDA strain 86. The fermentation tests revealed that this *Bacillus* is a great producer for the biosurfactant. The tests also displayed that the papaya peel extract is a

viable substrate for the production of biosurfactant by this strain. Among the results found, in 24 hours of cultivation, the highest concentration of biomass and product was obtained, of 2.17 ± 0.04 $g.L^{-1}$ and 2.88 ± 0.01 $g.L^{-1}$, respectively. The biosurfactant provided a Critical Micellar Concentration (CMC) of 20 $mg.L^{-1}$. The batch method was used in the obtainment of removal data, in which a series of solutions at different concentrations of iron ions were exposed to different amounts of biosurfactant, both raw and purified, at a temperature of 25 °C, under agitation (200 rpm) and pH ~ 6.3. A multivariate experimental design was carried out in the presence of crude and purified biosurfactant. The results demonstrated significant interactions involved for the following independent variables: concentration of iron ions, concentration of biosurfactant and the treatment time. The iron removal percentages varied between 47.2% and 95.82% in the presence of the raw biosurfactant, and between 37.01% to 91.94% in the presence of the purified surfactant. The Langmuir adsorption model was the better adjusted, providing a maximum adsorption capacity at approximately 10 $mg.g^{-1}$.

Keywords: *Bacillus subtilis*; Biosurfactant; Adsorption; Iron.

Resumen

La presencia de metales en los efluentes industriales se ha convertido en un problema ambiental importante ya que estos residuos a menudo se eliminan en lagos o ríos. Con el objetivo de recuperar áreas contaminadas, la remediación mediante el lavado con biosurfactantes aparece como una técnica alternativa que presenta baja toxicidad para el medio ambiente. El presente trabajo tuvo como objetivo evaluar la eficiencia en la eliminación de hierro en un efluente sintético, utilizando un biosurfactante producido en un biorreactor (37°C, 200 rpm, 0.5 vvm), del extracto acuoso de la cáscara de papaya y la cepa de *Bacillus subtilis* UFPEDA 86. Las pruebas fermentativas mostraron que este *Bacillus* es un buen productor de biosurfactantes y que el extracto de la cáscara de papaya es un sustrato viable para la producción de biosurfactantes por esta cepa. Entre los resultados encontrados, en 24 horas de cultivo, se obtuvo la mayor concentración de biomasa y producto, de 2.17 ± 0.04 $g.L^{-1}$ y 2.88 ± 0.01 $g.L^{-1}$, respectivamente. El biosurfactante producido mostró una concentración micelar crítica (CMC) de 20 $mg.L^{-1}$. S El método por lotes se utilizó en la obtención de datos de eliminación, en el que una serie de soluciones a diferentes concentraciones de iones de hierro se expusieron a diferentes cantidades de biosurfactantes, tanto crudos como purificados, a una temperatura de 25 ° C, bajo agitación (200 rpm) y pH ~ 6.3. E llevó a cabo una planificación experimental multivariada, en presencia de biosurfactante crudo y purificado, donde los resultados mostraron que las interacciones entre

las variables independientes (concentración de iones de hierro, concentración de biosurfactante y el tiempo de tratamiento) fueron significativas para ambos. Los porcentajes de eliminación de hierro variaron entre 47.2 y 95.82%, en presencia del bisurfactante crudo y de 37.01 a 91.94% en presencia del biosurfactante purificado. El modelo de adsorción Langmuir fue el mejor ajustado, con la capacidad de adsorción máx. estimada en 10 $mg.g^{-1}$.
Palabras clave: *Bacillus subtilis*; Biosurfactante; Adsorción; Hierro.

1. Introdução

Over the past decade, the contamination of water bodies by heavy metals has become a serious environmental concern the inappropriate, effluent disposals contaminated with metal ions, even in small amounts, can cause serious harm to both individuals and the ecosystem (Batista et al. 2020). Thus, these effluents must be treated to reduce the heavy metals to acceptable levels (Aguiar et al., 2002).

The treatment of wastewater involves the use of traditional methods, which include chemical precipitation and filtration, electrochemical treatment, oxidation or reduction, ionic exchange, and evaporation. However, these technologies present many disadvantages, such as the high costs, generation of pollutants and, above all, they are inefficient at removing heavy metal ions at low concentrations (Yildiz et al., 2017).

Faced with these problems, the process of heavy metal removal by washing using biosurfactants appears as an option that, besides being environment-friendly, allows the recovery of those metals for industrial ends and other applications (Jayabarath et al., 2009).

Numerous studies have presented the efficiency of biosurfactants in removing metals, both from soil and contaminated effluents. Juwarkar et al. (2008) studied the action of di-rhamnolipides, synthesized by *Pseudomonas aeruginosa* BS2, in the removal of metals. Juwarkar and co-authors observed that crude biosurfactant could remove chromium, zinc, copper and cadmium from a system infested with contaminates. Gnanamani et al. (2010) studied the bioremediation of Cr (VI), using the produced biosurfactant by the Bacillus. Sp. MTCC 5514. They observed that bioremediation involved two stages: the reduction of Cr (VI) to Cr (III). This is less toxic, as extracellular metabolites were synthesized by the strain and interactions were involved between the biosurfactant and the metal. Luna et al. (2016) studied the performance of an anionic biosurfactant, which originated from the *Candida sphaerica* strain. This included its heavy metal removal from soil collected at an automotive battery industry, in which removal rates of 95.90 and 79% for Fe were obtained, Zn and Pb,

respectively.

Biosurfactants are surfactants produced by microorganisms and which can capture metal ions by electrostatic interactions. The presence of functional groups of different polarities in their molecules leads to an interaction with the electric charge of the metals. Therefore, biosurfactants can act as a chelating agent and can enable the decontamination of soils and effluents (Soussi et al., 2019).

The development of large-scale heavy metal remediation techniques (remediation of large areas) employing biosurfactants requires efforts for the creation of new application technologies and production processes and the improvement of lineages. Biosurfactants may demonstrate economic benefit, yet due to the high costs involved in their production, purification and recovery processes, they are unable to compete with synthetic surfactants. However, a partial solution to this concern may be the use of alternative low-cost, highly available nutrient sources (Chooklin et al., 2014).

Therefore, this work intended to produce biosurfactant in a bioreactor from the strain *Bacillus subtilis* UFPEDA, and to assess its efficiency at the removal of iron from a synthetic effluent, using papaya peel as substrate, since, in a previous study (Soares, 2018), it proved to be an excellent source of carbon for the production of biosurfactant. In the end, a multivariate experimental planning was employed, where the following variables were used: metal concentration, biosurfactant concentration and reaction time.

2. Metodology

The research aims to search for new knowledge for society, as stated by Pereira et al. (2018). The present study is laboratory and quantitative in nature.

2.1. Microorganism maintenance

B. subtilis UFPEDA 86 used in this study was provided by the Department of Antibiotics of the Federal University of Pernambuco, Brazil. The strain was maintained in Luria-Bertani agar medium, as proposed by Cold Spring Harbor protocols (2010), with modifications. The modified medium was composed of 10.0 $g.L^{-1}$ tryptone, 5.0 $g.L^{-1}$ of yeast extract, 5.0 $g.L^{-1}$ NaCl and 20.0 $g.L^{-1}$ agar. The pH of the medium was adjusted to 6.8 using 1 M NaOH or 1 M HCl. The inoculation was performed in a laminar flow chamber and the

tubes were incubated at 37°C for 24 hours and then stored at 4°C. This procedure was repeated monthly for strain maintenance.

2.2. Pre-inoculum and inoculum

The broths used as pre-inoculum and inoculum, proposed by Bugay (2009) modified had the same composition: 20.0 g L^{-1} glucose, 3.0 g L^{-1} KH_2PO_4, 7.0 g L^{-1} K_2 HPO_4, 0.2 g L^{-1} $MgSO_4.7H_2O$, 1.0 g L^{-1} $(NH_4)_2SO_4$ and 1.0 g L^{-1} of yeast extract, pH corrected to 6.8 using 1 M NaOH or 1 M HCl. The pre-inoculum was prepared by transferring three culture loops to a 125 mL Erlenmeyer flask containing 30 mL of the medium and then brought to an incubator under orbital shaking (Tecnal® TE-424) at 37°C and 200 rpm for six hours. For the inoculum, 250 mL Erlenmeyer flask containing 50 mL of the medium was used. At this stage, an aliquot corresponding to 10% (v/v) of the medium (5 mL) was withdrawn from the pre-inoculum and transferred to the Erlenmeyer flask which was also incubated under orbital shaking at 37°C and 200 rpm for approximately 16 hours.

2.3. Substrate preparation

The papaya (*Carica papaya L.)* used in this work was obtained from a supermarket located in the city of Salvador, Bahia, Brazil. The edible parts were separated for consumption and the peels were homogenized with distilled water in a 2 L domestic blender (Philips® 500 W) at a concentration of 250 g.L^{-1} (Souza et al., 2012). Then, they were filtered and centrifuged (Hitachi Ltd. CR22G III) at 13.000 rpm at 4°C for 30 minutes until all the solid particles were removed and the aqueous extract (broth) used as substrate in the fermentation was obtained. The pH was corrected to 6.8 using 1 M NaOH or 1 M HCl.

2.4. Biosurfactant production

In this stage, the methodology adopted by Soares (2018) was followed, with modifications. The biosurfactant production process was conducted in a mechanically aerated and agitated bioreactor, with an effective volume of 4.5 L containing 3 L of broth obtained from a papaya peel that had been inoculated with 10% (v/v) of inoculum. The cultures were maintained at 37°C. Agitation and aeration conditions were kept in 200 rpm and 0.5 vvm for 144 hours. The foam formation was not controlled. Samples were collected in a regular time

interval to monitor the biomass concentration, substrate concentration, and product concentration. The sampling intervals were: two in two hours in the first six hours and then 24 in 24 hours until 144 hours of culture was completed. The samples were collected and centrifuged at 13.000 rpm at 4°C for 20 minutes to separate the biomass from the supernatant (cell-free fermented broth), which contained the biosurfactant.

2.5. Obtainment of crude surfactin extract

First, it was made the supernatant acid used HCl 3M until it reached a pH of 2.0. The acid supernatants were kept at a temperature of 4°C for 24 hours to allow precipitation of the biosurfactant. The precipitates were then separated by centrifuging at 10.000 rpm, 25 °C for 30 minutes in tubes that were previously weighed and labeled. After, they were dried at 30 °C for 24 hours in stove (Tecnal® TE-392/2) (Ghojavand et al., 2008).

2.6. Obtainment of purified surfactin extract

The raw surfactant extract solution was transferred to a separation funnel and subjected to three extraction stages. In the first stage, dichloromethane (1:1) was used, in the second, dichloromethane, chloroform and methanol (1:1:1:1) and, finally, in the third stage, chloroform and methanol (1:1:1). Each stage comprised of five minutes of agitation and one hour of rest for separation to occur, where the denser phase was retained in the funnel. After the three extraction stages, the organic phase was transferred to a tube and centrifuged at 13.000 rpm for five minutes. The supernatant was discarded, and the precipitate was dried at 30 °C for 24 hours (Ghojavand et al., 2008).

2.7. Determination of cell concentration

The cell concentration was determined by the dry mass method (Tríboli, 1989). In this method, 50 mL of the samples were transferred to pre-weighed tubes and then centrifuged at 13,000 rpm at 4°C for 20 minutes. The pellet formed was used to determine the cell concentration and the supernatant was separated for further analysis. Pellet was washed with distilled water and centrifuged three times to remove residues from the supernatant. After washing, the samples were placed in a drying oven (Tecnal® TE-392/2) at 65°C for 24 hours until constant weight. After this time, the tubes were placed in a desiccator for five minutes

and weighed. The biomass concentration ($g.L^{-1}$), [X], was expressed according to Equation 1. Where, m_{dry} = mass of the tube with dry biomass (g), m_{empty} = mass of the empty tube (g).

$$X = \frac{m_{dry} - m_{empt}}{50} \; x \; 1000 \quad (1)$$

2.8. Determination of product concentration

The product concentration ($g.L^{-1}$), [P], was expressed according to Equation 2 (Soares, 2018). Where, $m_{product}$ = mass of the tube with the precipitated biosurfactant after drying (g), m_{empty} = mass of the empty tube (g).

$$P = \frac{m_{product} - m_{empt}}{50} \; x \; 1000 \quad (2)$$

2.9. Determination of the surface tension

The surface tension of the cell-free fermented broth was monitored for 0, 2, 4, 6, 24, 36, 48, 60, 72, 84, 96, 120 and 144 h by the Du Noüy ring method using tensiometer (Kruss K20) at temperature of ± 25°C (Kuyukina et al., 2001).

2.10. Determination of critical micelle concentration (CMC)

Different concentrations of the cell-free fermented broth containing surfactin produced were obtained by performing several dilutions of this broth in distilled water (Santa Anna et al., 2002). The broth sample containing the highest concentration of surfactin was analyzed. Surface tension of the resulting solutions was measured at 25°C, as described previously. The CMC was determined by plotting the surface tensions ($mN.m^{-1}$) as a function of the concentration ($mg.L^{-1}$) and it was found at the intersection point between the two lines that best fit the pre- and post-CMC data (Gudina et al., 2010).

2.11. Preparation of the iron (II) solution

The iron-contaminated water was prepared by dissolving $FeNH_4(SO_4)_2.12H_2O$ in distilled water at a concentration of 1.000 $mg.L^{-1}$. This solution was later diluted according to the required concentration.

2.12. Iron (II) removal

According to Aguiar et al. (2002), the batch method was used in the obtainment of removal data, in which a series of solutions (20 mL) at different concentrations of metal ions were exposed to different amounts of biosurfactant, both raw and purified, at a temperature of 25 °C, under agitation (200 rpm) and pH ~ 6.3. The pH of the metallic solution was adjusted using NaOH (0.1 mol.L^{-1}) and was not correct along the adsorption experiments. The samples were all analyzed in an atomic absorption spectrophotometer (Shimadzu Corp. AA6030). After this measurement, the removal percentage was calculated by using Equation 3, where C_i is the starting iron concentration and C_f is the final concentration:

$$Extraction(\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (3)$$

2.13. Experimental planning

The software STATISTICA 7.0 was used in the statistical analysis and modeling of the response surface to assess the influence of the crude and purified surfactant on the heavy metal (iron) removal efficiency in a synthetic effluent. Therefore, two factorial designs (three factors and two levels) were used, which included three repetitions at the central point to assess the pure error, adding up eleven tests of each, which were randomly executed (Rodrigues & Iemma, 2014). The following variables were used: metal concentration, biosurfactant concentration and reaction time are presented in Table 1. The response variables were the Fe extraction percentage in the presence of crude surfactant or purified surfactant.

Table 1. Values used in the factorial planning for evaluation of the removal efficiency using biosurfactant.

Variables	**Levels**		
	-1	0	+1
Metal (mg.L^{-1})	20	35	50
Biosurfactant (mg.L^{-1})	CMC	2xCMC	5xCMC
Time (min)	2	5	8

Fonte: Authors.

In the present study, the levels for each variable were chosen according to the following criteria: the metal concentration, time interval, and biosurfactant concentration,

which were defined according to data obtained in the literature (Brasil et al., 2007, Buratto et al., 2012, Almeida et al., 2018).

The effects of the variables and the respective errors were calculated at a 95% confidence limit.

2.14. Iron (II) adsorption isotherms

The equilibrium study for the adoption process helps to determine if the absorbent is able for cleaning the solution. This measures the maximum adsorbate amount that an adsorbent can take in (Almeida & Santos, 2020).

As proposed by Colla et al. (2012), the batch method was used to obtain the adsorption data, where a series of solutions (20 mL) at different concentrations of metallic ions were exposed to a certain amount of adsorbent (0.05 g), at a temperature of 25°C, under agitation (200 rpm) and pH ~ 6.3. The pH of the metallic solution was adjusted by applying NaOH (0.1 $mol.L^{-1}$) and was not corrected during the adsorption experiments. A buffer solution was not used so as not to increase the competitive effect of the different species to the adsorption site. The starting concentration of the metallic ion varied from 20 to 50 $mg.L^{-1}$. All the tests were repeated three times. After 24 hours of agitation to reach balance, the samples were centrifuged at 5000 rpm for 10 minutes and then filtered in black ribbon paper. The concentrations of the metals were then measured by Atomic Absorption Spectrometry (AAS). The metallic ion numbers adsorbed by the biosurfactant were calculated using Equation 4.

$$q = \frac{V(C_i - C_f)}{M} \quad (4)$$

Where C_i is the initial metal concentration, C_f the equilibrium metal concentration, M is the biosurfactant mass and the volume of the metal ion solution.

The Langmuir and Freündlich isotherm models were applied to the obtained data. The mathematical expressions that represent their isotherms are defined by equations 5 and 6, respectively:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad (5)$$

Where, q_e is the amount of solute adsorbed per gram of adsorbent at equilibrium (mg.g^{-1}), q_{max} the maximum adsorption capacity (mg.g^{-1}), K_L the Langmuir equilibrium constant (L.mg^{-1}) and Ce the concentration of adsorbate at equilibrium (mg.L^{-1}).

$$q_e = K_F C_e^{\frac{1}{n}} \quad (6)$$

Where, q_e is the amount of solute adsorbed per unit mass (mg.g^{-1}), K_F the Freundlich adsorption capacity constant, C_e the equilibrium concentration in solution (mg.L^{-1}) and $\frac{1}{n}$ the constant related to the heterogeneity of the surface.

3. Resultados e Discussão

3.1. Cell concentration and product concentration

Table 2 displays the cell concentration and product concentration formed along 144 hours of fermentation.

Table 2. Values of cell concentration and product concentration along 144 hours of fermentation.

t (h)	X (g/L)	P (g/L)
0	0.07 ± 0.02	0.63 ± 0.01
2	0.24 ± 0.01	0.71± 0.01
4	0.34 ± 0.01	0.89 ± 0.08
6	0.42 ± 0.03	0.93± 0.05
24	2.17 ± 0.04	2.88 ± 0.01
48	2.09 ± 0.01	2.67 ± 001
72	1.90 ± 0.08	2.59 ± 0.02
96	1.70 ± 0.07	2.66 ± 0.03
120	1.40 ± 0.05	2.77 ± 0.08
144	1.19 ± 0.04	2.73 ± 0.01

*t – Time of fermentation, X – Cell concentration, P – Product concentration.

Examining Table 2, it is possible to verify that at the begging of fermentation the cell concentration was 0.07 ± 0.02 g.L^{-1} and the maximum cell concentration observed was 2.17 ± 0.04 g.L^{-1}, in 24 hours of cultivation.

Pinto et al. (2009) studied the production of biosurfactants in four microorganism cultures, including a pure culture of *Bacillus subtilis*, for 72 hours, at 200 rpm and 30 ° C in synthetic medium. Among other compounds, 40 $g.L^{-1}$ of glucose were carbon sources. Pinto and co-authors obtained a maximum cell concentration of 0.96 $g.L^{-1}$, which is lower than what is presented in this study. This suggests that the substrate synthesized from papaya peels is a rich medium and can easily be replaced by synthetic media. This would reduce production costs and contribute to a decline towards environmental pollution.

Soares (2018) studied the production of biosurfactant by the *Bacillus subtilis* UFPEDA 86. The submerged fermentation was carried out in a rotary orbital shaker at 37 ° C, 200 rpm for 96 hours. The maximum cell concentrations obtained for the three media occurred within 36 hours of culture. The values were 0.83 ± 0.01 $g.L^{-1}$, 1.07 ± 0.08 $g.L^{-1}$ and 0.14 ± 0.02 $g.L^{-1}$ for glucose, papaya peel aqueous extracts and passion fruit peel aqueous extracts, respectively. Soares observed that the microorganism developed better within the substrate composed of papaya peels in comparison to glucose and did not develop well for the substrate surrounding the passion fruit peels, which obtained a lower concentration than glucose. The author mentioned that higher cell concentrations within the complex medium composed of papaya peels when compared to the medium composed of glucose may have been influenced by the macro and micronutrients present. This provides better nutritional balance and may have favored the growth of the *Bacillus*. For all the media used, including the media containing papaya peel extract (same substrate used in this study), the values of maximum cell concentrations obtained by Soares (2018) were lower than the values in this study. For this review, fermentation was carried out in a bioreactor, where it provided greater aeration for the medium and homogenization within the substrate. This facilitates the consumption of nutrient sources by microorganisms and can allow greater growth for these microorganisms.

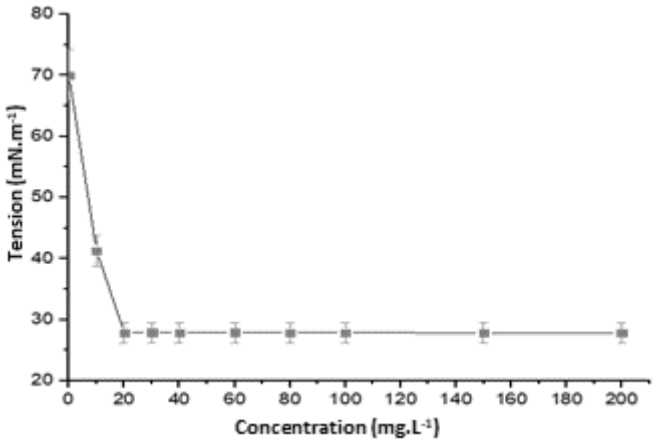
The product formation analysis revealed that the greatest concentration of crude surfactin obtained was 2.88 ± 0.01 $g.L^{-1}$ in 24 hours. For surfactin production, the results are superior in comparison to those found by different authors. Liu et al. (2012) cultured *Amyloliquefaciens* in medium using sucrose as substrate and obtained 0.13 $g.L^{-1}$ surfactin in 48 h culture time. Oliveira (2010) produced surfactin by *B. subtilis* LAMI005 in a medium composed of cashew berry juice and obtained 0.21 $g.L^{-1}$ in 48 hours of culture. And Al-Bahry & Al-Wahaibi (2013), using *Phoenix dactylifera L.* as the only source of carbon and energy for the production of biosurfactant by *B. subtilis* B20, obtained concentrations of 2.29 $g.L^{-1}$ in 10 hours of cultivation.

3.2. Determination of critical micellar concentration (CMC)

An important property of surfactants is their efficiency for tension reduction, both for superficial or interfacial, and the Critical Micellar Concentration (CMC), which is a widely used index to assess surfactant activity (Medeiros, 2007). The CMC is the minimum required concentration to achieve lower surface tension values, at which the formation of micelles starts (Banat et al., 2000).

The CMC was determined by the measurement of the surface tension for various biosurfactant concentrations. In the CMC tests, the supernatant samples from the fermentation broth obtained the highest formation of surfactin (24 hours of fermentation), which are shown in Figure 1.

Figure 1. Variation of surface tension at different concentrations of biosurfactant.



Source: Authors.

Figure 1 illustrate an increase in the concentration for the resulting biosurfactant which led to a reduction of the surface tension of distilled water until the CMC was reached at approximately 20 $mg.L^{-1}$. In spite of the increase in the surfactin concentration, there was no reduction of the superficial tension. The raw biosurfactant produced by *B. subtilis* UFPEDA 86 presented great reduction capability for the superficial tension since, with just 20 $mg.L^{-1}$, it could reduce the superficial tension of water from 70.14 ± 0.03 to 27.85 ± 0.02 $mN.m^{-1}$, causing tension reductions of 60.29 ± 0.03%.

The biosurfactant displayed lower critical micellar concentration than the biosurfactants produced by *B. subtilis* in studies conducted by Kim et al. (1997) and Vaz et al. (2012) as they utilized glucose and raw petroleum media as substrate, respectively, and

obtained CMC of 40 mg.L^{-1}. On the other hand, Oliveira et al. (2013) achieved CMC of 10 mg.L^{-1} using *Bacillus pumilus* and wheat bran as an alternative substrate. Feliz (2012), produced biosurfactant from *Bacillus subtilis* LAMI005 and cashew berry juice, reported CMC of 12 mg.L^{-1}. Similar results in this study was obtained by Barros et al. (2008), with a CMC of 19 mg.L^{-1} with the production of raw surfactin by *Bacillus subtilis* LB5a using cassava residue as substrate. Such variations in CMC values for surfactin have been commonly described by other authors who explained that such changes depend on the nature of the solvent used to dissolve surfactin as well as the purity of the surfactin preparation. From the results obtained in the present study, 20 mg.L^{-1} are within the range obtained by the aforementioned author and are in agreement with results more commonly described in literature ranging from 1 to 200 mg.L^{-1} according to Costa (2005), which shows the potential of using papaya (*Carica papaya L.*) wastes as a carbon source for the production of surfactin by *B. subtilis* UFPEDA 86.

3.3. Experimental planning

Table 3 presents the experimental matrix and the removal percentage results, which were obtained through factorial planning.

Table 3. 2^3 Factorial Experimental Planning matrix for Fe using the raw and purified surfactant.

Test	[Me] (mg.L^{-1})	[B] (mg.L^{-1})	t (min)	ERb (%)	ERp (%)
1	20	CMC	2	55.95	56.94
2	50	CMC	2	49.97	67.38
3	20	5xCMC	2	47.2	49.61
4	50	5xCMC	2	95.82	85.85
5	20	CMC	5	72.5	86.06
6	50	CMC	5	64.94	69.68
7	20	5xCMC	5	48.97	37.01
8	50	5xCMC	5	80.93	86.01
9	35	2xCMC	8	51.92	90.09
10	35	2xCMC	8	49.42	91.94
11	35	2xCMC	8	50.82	88.45

*[ME] – Fe concentration, [B] – Biosurfactant concentration, t – Time of treatment, ERb – Raw biosurfactant removal efficiency, ERp – Purified biosurfactant removal efficiency.

According to Table 3, the presence of raw surfactant increased the removal efficiency when compared to the purified biosurfactant, where the removal percentages varied between 47.2 and 95.82% in the presence of the raw biosurfactant, and from 37.01 to 91.94% in the presence of the purified surfactant.

Yuan et al. (2008), managed to remove 89.95% of lead, 81.13% of copper and 71.17% of cadmium from an aqueous solution using saponins. Mulligan et al. (2001) demonstrated that raw surfactin could remove 70% of copper and zinc ions from a contaminated soil sample. Ramani et al. (2012) using the biosurfactant produced by *Bacillus circulans*, achieved removal percentages for both Fe^{2+} and Cu^{2+} at 57.5% and 69%, respectively. Das, Mukherjee & Sen (2009) reported similar results in this study as they utilized purified biosurfactant by *B. subtilis* LAMI005, where they managed to remove 89% of Fe and 98% of Cu from an aqueous solution.

Tables 4 and 5 present the main effects and interaction effects of the independent variables for iron using the raw and the purified biosurfactant, respectively, having as response the removal percentage for this metal.

Table 4. Estimated effects of the central composed planning for iron using raw biosurfactant.

	Effect	**Pure error**	**t (2)**	**P**	**Confidence limit - 95%**	**Confidence limit + 95%**
(1)[Me] ($mg.L^{-1}$)	16.760	0.886	18.916	0.003	12.948	20.572
(2)[B] ($mg.L^{-1}$)	7.390	0.886	8.341	0.014	3.578	11.202
(3) t (min)	4.600	0.886	5.192	0.035	0.788	8.412
1 x 2	23.530	0.886	26.557	0.001	19.718	27.342
1 x 3	-4.560	0.886	-5.147	0.036	-8.372	-0.748
2 x 3	-11.160	0.886	-12.596	0.006	-14.972	-7.348

*[Me]: Fe concentration, [B]: Biosurfactant concentration, (t): Time of treatment, 1 x 2: [Me] x [B] interaction, 1x3: [Me] x T interaction and 2x3: [B] x T interaction.
Source: Authors.

Table 5 – Estimated effects of the central composed planning for iron using purified biosurfactant.

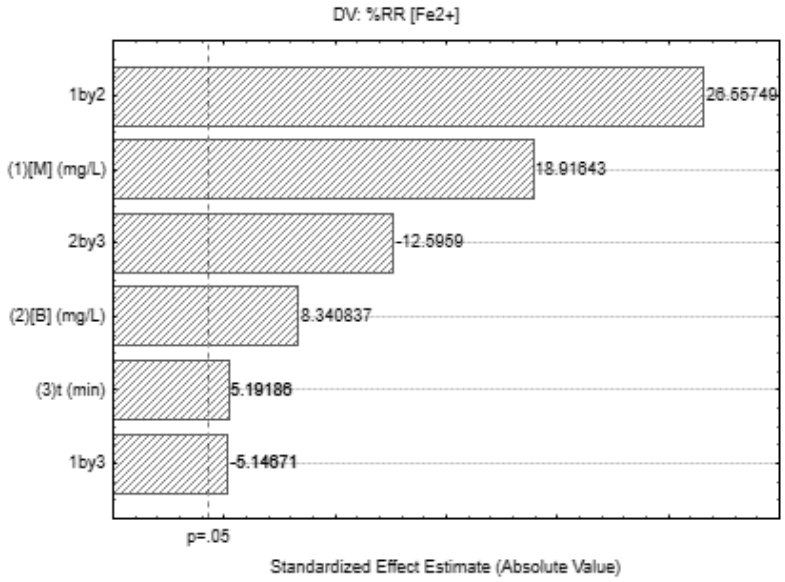
	Effect	Pure error	t (2)	P	Trust limit - 95%	Trust limit + 95%
(1)[Me] ($mg.L^{-1}$)	20.325	1.235	16.462	0.004	15.013	25.637
(2)[B] ($mg.L^{-1}$)	-4.895	1.235	-3.965	0.048	-10.207	0.417
(3)t (min)	5.245	1.235	4.248	0.041	-0.067	10.557
1 x 2	27.295	1.235	22.108	0.002	21.983	32.607
1 x 3	-9.015	1.235	-7.302	0.018	-14.327	-3.703
2 x 3	-6.465	1.235	-5.236	0.035	-11.777	-1.153

*[Me]: Fe concentration, [B]: Biosurfactant concentration, (t): Time of treatment, 1 x 2: [Me] x [B] interaction, 1x3: [Me] x T interaction and 2x3: [B] x T interaction.

Tables 4 and 5 exhibit the interactions between factors that were statistically significant ($p < 0.05$). Therefore, the effects of the factors did not must interpret separately. Both tables demonstrated through the 1x2 interaction that the increase in the biosurfactant concentration from CMC to 5xCMC increased the removal of iron ions when it was increased its concentration from 20 to 50 $mg.L^{-1}$. As observed through the 1x3 interaction, the removal of iron ions decreased when its concentration in the effluent and the treatment time increased. Finally, by increasing the treatment time from 2 to 8 min and increasing the biosurfactant concentration from CMC to 5xCMC, there was a decrease in the removal of iron ions in the effluent (2x3 interaction).

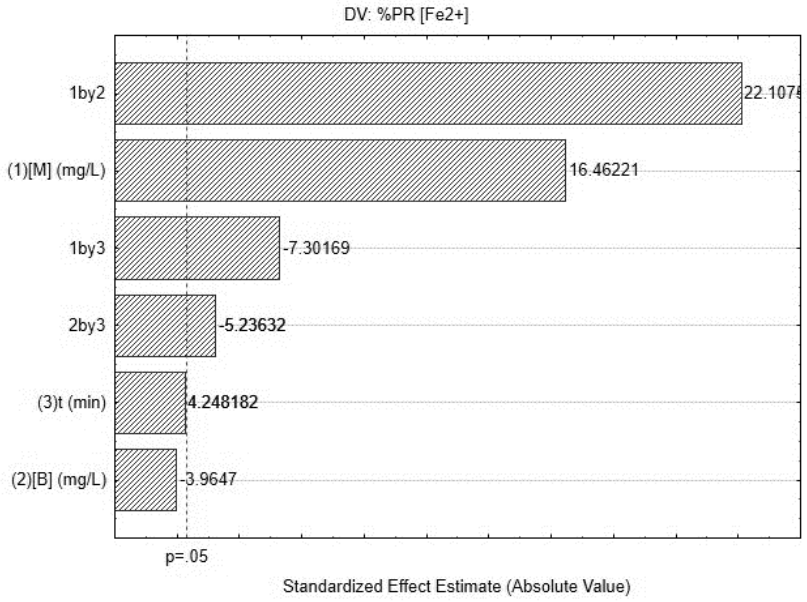
Figures 2 and 3 show the Pareto chart of the factorial design for iron, using the raw and purified surfactants. This is one of the forms of visually evaluate the influence of the factors in the response. The magnitude of the effects is represented by the horizontal columns, whereas the transverse line represents the magnitude of the effects with statistical significance for p=0.05, that is, the factors are statistically significant at the 95% trust interval.

Figure 2. Pareto chart of the factorial planning for iron using the crude biosurfactant.



Source: Authors.

Figure 3. Pareto chart of the factorial planning for iron using the purified biosurfactant.



Source: Authors.

When the interaction effects are taken into account, the synergistic effect of the iron concentration and the biosurfactant concentration had a greater influence on the metal removal efficiency, both in the presence of the raw biosurfactant and in the presence of the

purified surfactant (Figures 2 and 3). As shown in Figure 2, the biosurfactant concentration and the time together had a negative and antagonistic effect on the metal removal efficiency, being this same behavior observed in the presence of the purified surfactant (Figure 3), but with less influence. In turn, the metal concentration and time, for both conditions understudy, had an antagonistic effect on the efficiency, with a greater influence in the presence of the purified surfactant.

3.4. Fe adsorption by the resulting biosurfactant

In Table 6, the adsorption parameters were shown.

Table 6. Adsorption parameters of the Langmuir and Freündlich isotherms for Fe and the resulting biosurfactant.

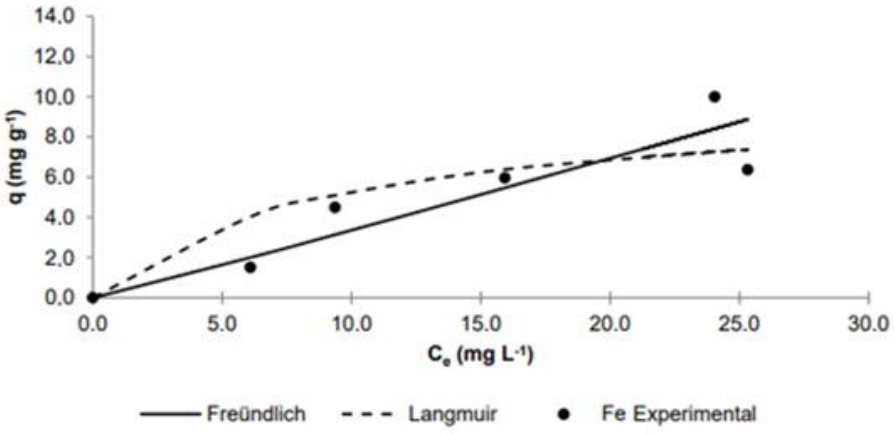
Parameters	**Langmuir**			**Freündlich**		
	K_L	B	R^2	K_F	1n	R^2
	$L.mg^{-1}$	$mg.g^{-1}$		$L.mg^{-1}$		
	0.25	10.00	0.9981	100.79	1.21	0.8710

*K_L – constant related to the affinity of the adsorbent to the adsorbate, b – maximum adsorption capacity, R^2 – determination coefficient, K_F - Freündlich's adsorption constant.

The high correlation coefficients obtained from the Langmuir and Freündlich linearized equations (Table 6) suggest that both adsorption models are significantly capable of demonstrating the Fe adsorption by the resulting biosurfactant.

Figure 4 illustrates the experimental data and the isotherm obtained by the Langmuir and Freündlich models for the adsorption of Fe by the biosurfactant. The value of b (10 $mg.g^{-1}$) obtained by Langmuir's isotherm estimates the Maximum Adsorption Capacity (MAC) and the result suggests that this biosurfactant has an elevated affinity for the metal under study.

Figure 4. Isotherm obtained from the adjustment of the adsorption equations to the data experimentally obtained for iron.



Observing the experimental values, regardless of the fit or unfit for the evaluated physicochemical models, these results are necessary in confirming the resulting biosurfactant, produced from the papaya peel residue, which is capable of adsorbing metals.

Some studies already reported in the literature, also showed the good performance of biosurfactants in the removal of metal ions in contaminated effluents. Valdman et al. (2005) demonstrated that an exopolysaccharide extracted from the microorganism *Serratia sp*, with biosurfactant activity, showed high efficiency in removing Cd from liquid media. The experiments were conducted with 50 $mg.L^{-1}$ of Cd^{2+} and 0.1 $g.L^{-1}$ of the previously purified biosurfactant, resulting in removals in the order of 170 mg of Cd g^{-1} of biosurfactant. Colla et al. (2012) in a study that compared the removal of Cd in liquid medium containing biosurfactant and different species of filamentous fungi, concluded that in those media containing biosurfactant, the removal of metal was greater, achieving 100% in the presence of *Aspergillus* fungi. The authors concluded that the presence of biosurfactants in liquid media improves the efficiency of metal removal in liquid media.

The biosurfactant´s capacity of adsorbing metals is due to its anionic nature, which allows the interaction between negative charges to be present in the compound and the positive charges to be shown in the metal ions. Moreover, it is important to note that this anionic nature is due to the presence of functional groups such as carboxylic acids and amino groups (Franzetti et al., 2014). This behavior corroborates the study by Das et al. (2009), where they studied the synthesis of biosurfactant utilizing isolated bacteria derived from a marine environment. Das and co-authors evaluated its potential in reducing metal contamination present in industrial effluents. They observed that synthesized biosurfactants

obtained anionic characteristics and were able to interact with the metals by ionic bonds. In addition, a precipitate was formulated (metal-biosurfactant complex), which can be simply removed from the solution by unit centrifugation operation.

4. Considerações Finais

The fermentation tests conducted in the bioreactor presented an aqueous extract of papaya peel which obtains a great carbon source for the production biosurfactants by *Bacillus subtilis* UFPEDA 86. In 24 hours of cultivation, the largest concentration of biomass obtained was 2.17 ± 0.04 $g.L^{-1}$, whereas the surfactin yield was 2.88 ± 0.01 $g.L^{-1}$.

The critical micelle concentration (CMC) of biosurfactant was found to be 20 $mg.L^{-1}$.

The experimental designs allowed us to state that the presence of the raw biosurfactant increased the removal efficiency when compared to the purified biosurfactant, where the removal percentages varied between 47.2% and 95.82% in the presence of the raw biosurfactant, and between 37.01 to 91.94% in the presence of the purified surfactant.

In the experimental design, using raw or purified biosurfactant, there was a significant interaction found for the independent variables observed (iron ions concentration, biosurfactant concentration and time).

In this study, the Freudlich's adsorption model adjusted well to the iron, however, the Langmuir's model was better adjusted, being the maximum adsorption capacity estimated in 10 $mg.g^{-1}$.

The results showed that the biosurfactant produced can be used in the treatment of effluents contaminated by iron.

Conflict of Interest

The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals

This paper does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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