Vol. 17(30), pp. 928-937, 25 July, 2018
DOI: 10.5897/AJB2018.16408
Article Number: 37A9B8557987
ISSN: 1684-5315
Copyright ©2018
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Biosurfactant production by *Bacillus subtilis* UFPEDA 86 using papaya (*Carica papaya* L.) waste as substrate: Viability studies and pH influence of the culture medium

Camylla Carneiro Soares[1]*, Adrielly Silva Albuquerque de Andrade[2], Gabriela Fontes Deiró Ferreira[1], Andrea Farias de Almeida[2], Janice Izabel Druzian[1] and Ana Katerine de Carvalho Lima Lobato[1,3]

[1]Department of Chemical Engineering, Federal University of Bahia, 40210-910 Salvador, Bahia, Brazil.
[2]Department of Biotechnology, Federal University of Paraiba, 58051-900 João Pessoa, Paraiba, Brazil.
[3]Department of Chemical Engineering, Salvador University, 40231-902 Salvador, Bahia, Brazil.

Received 23 January, 2018; Accepted 8 June, 2018

Biosurfactants are surface-active compounds derived from microorganisms and offer several advantages over chemical surfactants, such as low toxicity, good biodegradability and ecological acceptability. Even though interest in biosurfactants is increasing, these bioproducts do not compete economically with synthetic surfactants due to the overall costs of the bioprocess. The use of inexpensive raw materials is an important approach to reduce these costs since the substrate price account for 10 to 30% of the final product expenses. In this study, papaya (*Carica papaya* L.) waste was used as a substrate to produce biosurfactant by *Bacillus subtilis* UFPEDA 86 strain. In addition, culture medium pH was corrected from 5.25 to 6.8 in order to analyze the influence of this variable on the biosurfactant production. The submerged fermentation was carried out on a shaker incubator at 37°C, 200 rpm for 96 h. Biomass and substrate concentration, surface tension, emulsification index and critical micelle concentration were analyzed. The strain was well adapted to both substrates studied, without and with pH correction. Using the broth without pH correction (pH=5.25), a maximum cell concentration of 1.07 g L^{-1} at 36 h a 25.5% surface tension reduction, emulsification index of around 61% and critical micelle concentration of about 35 mg L^{-1} was obtained. Using the broth with pH correction to 6.8, better results; maximum cell concentration of 1.14 g L^{-1} at 24 h, a 32.5% surface tension reduction, emulsification index of around 66% and critical micelle concentration at about 35 mg L^{-1} was obtained. The papaya waste proved to be an effective substrate in the biosurfactant production by *B. subtilis* UFPEDA 86 and the pH variable proved to be of great importance in the yield of this process.

Key words: Biosurfactant, submerged fermentation, papaya waste, *Bacillus subtilis*.

INTRODUCTION

Surfactants are amphipathic molecules, consisting of hydrophobic and hydrophilic portions, which act preferentially at the interface between fluid phases with different polarities, promoting the reduction of surface and interfacial tensions between immiscible compounds. Its amphiphilic capacity increases solubility and mobility of hydrophobic and organic compounds insoluble in aqueous solutions (Ghojavand et al., 2008; Singh et al.,

2007; Ying, 2006). Thus, surfactants are applied in a wide variety of industrial processes involving emulsification, foam, detergency, wetting, dispersion or solubilization (Nitschke et al., 2004).

Most of the surfactants produced are chemically derived from petroleum. However, increasing environmental concerns have led to the search of natural surfactants as an alternative to the traditional synthetic products (Barros et al., 2007). Natural surfactants synthesized by microorganisms are called biosurfactants and offer several advantages over chemical surfactants such as low toxicity, biodegradability, ecological acceptability, stability to extreme temperature, pH or salinity conditions and the possibility of production from renewable sources (Lobato, 2013; Rocha, 2017). In addition to these characteristics, biosurfactants have a wide variety of potential applications, which make them even more interesting. The main applications of these biological compounds are in the advanced oil recovery and in the bioremediation fields, however, they can still be used as emulsifiers, functional ingredient, microbiological, pharmaceutical and therapeutic agent, and as additives in health and beauty products (Ghojavand et al., 2008).

Biosurfactants have a wide variety of chemical structures, including glycolipids, lipopeptides, phospholipids, fatty acids or neutral lipids, among others (Geys et al., 2014; Gudiña et al., 2013). *Bacillus* species produce a broad spectrum of lipopeptide biosurfactants, which are cyclic molecules consisting of a variable-length fatty acid (hydrophobic fraction) bound to a short peptide chain (hydrophilic fraction) of seven or ten amino acids. Among them is the surfactin, a lipoheptapeptide produced by *Bacillus subtilis* strains and one of the most effective biosurfactants known so far (Singh et al., 2009).

In spite of the increasing interest in biosurfactants, these compounds do not compete economically with synthetic surfactants due to the high process costs associated with inefficient methods of bioproducts recovery and the use of costly substrates, which account for about 10 to 30% of the overall production cost (Lobato, 2013; Tuleva et al., 2009). A method to reduce such costs is the use of alternative substrates with a good balance of carbohydrates and lipids to provide efficient microorganism growth conditions and biosurfactant synthesis. In this sense, by-products of agroindustry activity are great substrate sources for biosurfactant production and their use can diminish the environmental problems caused by their inadequate disposal, as well as the costs associated with effluent treatments (Gallert and Winter, 2002; Makkar and Cameotra, 2002; Mukherjee and Das, 2005). Thus, the use of agroindustrial wastes brings value to these materials and contributes to the sustainability of their economical chain (Rocha, 2017).

Papaya (*Carica papaya* L.) peels, for example, provide a good alternative substrate for fermentation since they have a good nutritional balance and are usually discarded in the fruit processing industries (Gondim et al., 2005; Lima et al., 2008). According to Gondim et al. (2005), the main macronutrient present in the papaya waste is carbohydrate, which makes this residue an excellent energy source and a good substrate for fermentation. In addition, the peels of this fruit have pH around 5.5, which makes them less acidic when compared to the other fruits peels and therefore less aggressive to the microorganism. According to data provided by the quality control of Brasfrut® (Feira de Santana, BA, Brazil), about 65 tons of papaya wastes were eliminated by this company in 2016. Despite the good characteristics of the papaya waste and the sustainability involved in its use, no study is reported in the literature using these residues as a substrate for the biosurfactant production.

In this context, the present work aimed to evaluate the biosurfactant production by *Bacillus subtilis* strain UFPEDA 86 using papaya (*C. papaya* L.) waste from the industrial fruit processing as substrate, and to analyze if the pH modification of the culture medium is effective in the biosurfactant production. Cell growth, substrate consumption, surface tension reduction, emulsification index and critical micelle concentration were evaluated during 96 h of fermentation.

MATERIALS AND METHODS

Microorganism maintenance

B. subtilis UFPEDA 86 used in this study was kindly 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 h and then stored at 4°C. This procedure was repeated monthly for strain maintenance.

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_2HPO_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

*Corresponding author. E-mail: s.camylla@yahoo.com.br. Tel: +55 75 991422412.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

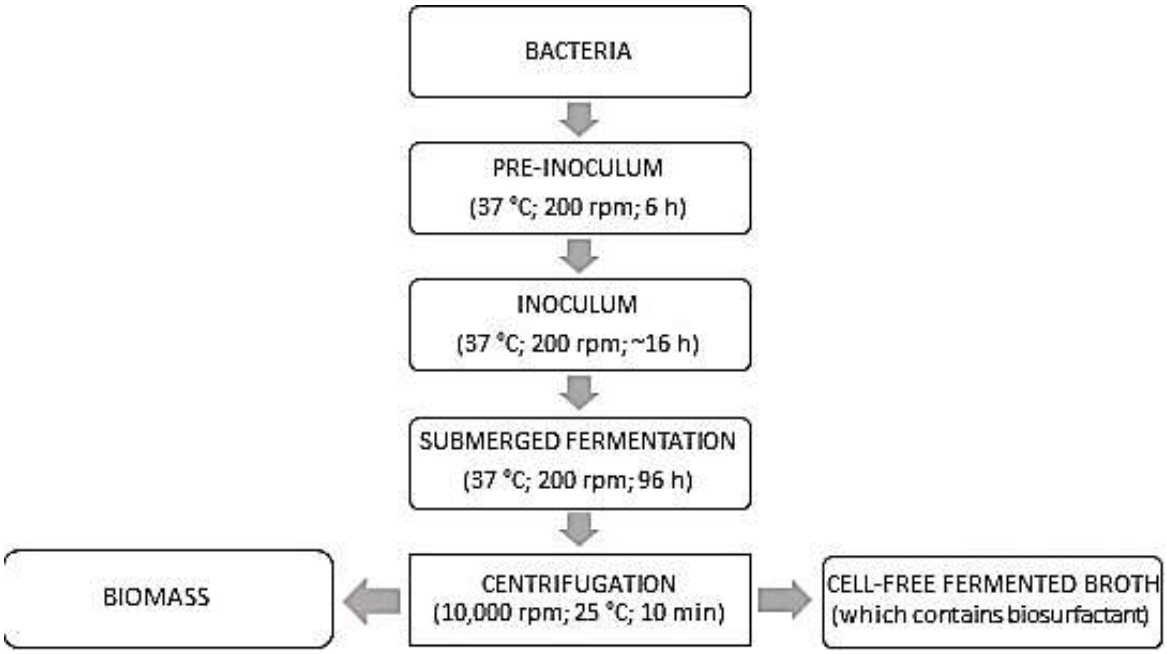

Figure 1. Biosurfactant production steps.

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 6 h. 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 inoculum (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 about 16 h.

Substrate preparation

The papaya (*Carica papaya* L.) waste used in this work was obtained from Brasfrut®, a fruit processing industry located in the city of Feira de Santana, Bahia, Brazil. The residues were homogenized with distilled water in a 2 L domestic blender (Philips® 500 W) at the concentration of 250 g L^{-1} (Souza et al., 2012). Then, they were filtered and centrifuged (Hitachi Ltd. CR22G III) at 10,000 rpm at 25°C for 10 min until the removal of all the solid particles and obtaining of the aqueous extract (broth) used as substrate in the fermentation. In order to check the influence of pH on the biosurfactant production, a similar medium with pH corrected to 6.8 was prepared.

Biosurfactant production

The production was carried out by submerged fermentation in an orbital shaker incubator at 37°C and 200 rpm during 96 h. Samples were collected at regular intervals to monitor the biomass concentration, substrate concentration, surface tension variation, emulsification index and critical micelle concentration. The sampling intervals were: 4 in 4 h in the first 12 h and then 12 in 12 h until complete 96 h of culture. The samples were collected and centrifuged at 10,000 rpm at 25°C for 10 min to separate the biomass from the supernatant (cell-free fermented broth), which contained the biosurfactant. Figure 1 shows, in a simplified way, the biosurfactant production steps.

Determination of biomass concentration and cell productivity

The biomass 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 10,000 rpm at 25°C for 10 min. The pellet formed was used to determine the cell concentration and the supernatant was separated for further analysis. The pellet was washed with distilled water and centrifuged 3 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 h until constant weight. After this time, the tubes were placed in a desiccator for 5 min and weighed. The biomass concentration (g L^{-1}), [X], was expressed according to Equation 1.

$$[X] = \frac{m_{dry} - m_{empty}}{50} \times 1000 \quad (1)$$

Where, m_{dry} = mass of the tube with dry biomass (g); m_{empty} = mass of the empty tube (g).

The cell productivity (Px; g L^{-1} h^{-1}) was determined by Equation 2 described by Schmidell et al. (2001).

$$P_X = \frac{X_m - X_o}{t_f} \quad (2)$$

Where, X_m = maximum cell concentration (g L^{-1}); X_o = initial cell concentration (g L^{-1}) and t_f = total time of the fermentation (h).

Determination of substrate concentration (sugars concentration)

The sugars quantification was performed by the 3,5-dinitrosalicylic acid (DNS) method, which determines reducing sugars, as

proposed by Miller (1959). The samples were initially hydrolyzed in test tubes by the addition of 1 mL of the supernatant and 0.5 mL of pure HCl. The tubes were taken to a water bath for 10 min at 70°C and then cooled on ice bath for approximately 5 min. After this period, 2 mL of 4 N NaOH was added and the tube content was completed to 10 mL using distilled water.

After hydrolysis, aliquots of 200 µL of the hydrolyzed samples were transferred to test tubes containing 200 µL of DNS reagent. The tubes were then brought to a water bath at 100°C for 10 min followed by ice bath for 5 min. After this procedure, it was added 5 mL of distilled water, followed by agitation in a vortex (Phoenix® AP56) and absorbance reading in a spectrophotometer (Shimadzu Corp. UV-1800) at 540 nm. A sample containing 0.5 mL of distilled water was used as blank in place of 0.5 mL of the hydrolyzed sample. A calibration curve was constructed by correlating the concentration of glucose and fructose with the absorbance reading.

Determination of the surface tension

The surface tension of the cell-free fermented broth was monitored for 0, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 h by the Du Noüy ring method using tensiometer (Kruss K20) at temperature of ± 25°C (Kuyukina et al., 2001).

Determination of emulsification index

Cell-free fermented broths were mixed to hydrophobic compounds - soy oil, gasoline and diesel - in test tubes in the ratio of 4:6, respectively, and homogenized in a vortex at full speed for 1 min. The tubes were allowed to stand at room temperature for 24 h and at the end of the time measurements were made of the height of the emulsified layer and total height of the liquids in the tube using a ruler. The emulsification index was obtained by Equation 3 described by Cooper and Goldenberg (1987).

$$EI_{24}\ (\%) = \frac{EL}{TH} \times 100 \quad (3)$$

Where, EI_{24} = emulsification index (%); EL = height of the emulsified layer (cm); TH = total height of the liquids (cm).

Determination of critical micelle concentration (CMC)

Different concentrations of the cell-free fermented broth containing surfactin produced after 24 h of fermentation were obtained by performing several dilutions of this broth in distilled water (Santa Anna et al., 2002). 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).

RESULTS AND DISCUSSION

Biomass concentration, cell productivity, substrate concentration and surface tension

The curves shown in Figure 2, plotted using Origin 8.1 (OriginLab CO., MA, USA), represent the cell growth (biomass), the substrate consumption and the surface tension behavior along 96 h of fermentation using the broth prepared from papaya (*Carica papaya* L.) waste as substrate without pH correction (Figure 2A) and with the pH adjusted to 6.8 (Figure 2B).

When analyzing the biomass curve in Figure 2A, the following stages of microbial growth are defined: Latency phase (lag phase) from 0 to 2 h; exponential phase (or log phase) from 2 to 36 h; stationary phase from 36 to 60 h and decline phase from 60 h.

The small lag phase demonstrates that the *Bacillus subtilis* UFPEDA 86, although usually cultivated in media containing glucose as the main carbon source, was easily adapted to the substrate used in this study, demonstrating the versatility of this microorganism when using more complex substrates.

Observing the biomass curve in Figure 2B, for fermentation with pH adjusted to 6.8, the following stages of microbial growth are defined: Latency phase (lag phase) nonexistent in the time interval analyzed; exponential phase (or log phase) from 0 to 24 h; stationary phase from 24 to 60 h and decline phase from 60 h.

The absence of the lag phase demonstrated that the microorganism was adapted almost instantaneously to the complex medium used in this work. This fast adaptation may be due to the change in the pH of the medium since the microorganism was cultivated at a pH close to neutrality and this one was maintained during fermentation. In this way, this variable showed important influence in terms of process optimization, once the exponential phase, which is associated with the formation of the bioproduct, was more quickly achieved.

The maximum cell concentration obtained using the broth without pH correction was 1.07 g L^{-1} at 36 h and 1.14 g L^{-1} using the broth with pH correction at 24 h, which shows that *Bacillus* was able to develop in both culture media proposed in this study. The biomass productivities, for the broths without pH correction and with 6.8 pH were 0.011 and 0.012 g L^{-1} h^{-1}, respectively. The difference in biomass productivities in the two fermentation configurations studied was very low. However, in the broth fermented at 6.8 pH, this parameter was reached 12 h earlier, compared to the broth with no pH adjustment. In this way, the industrial relevance of pH correction of the culture medium has once again been demonstrated, since there will be time savings in the formation of the bioproduct.

In terms of cell performance, Silva et al. (2015) studied the growth of *Bacillus subtilis* UFPEDA 86 for 48 h using glucose and sodium nitrate as carbon and nitrogen sources and obtained a maximum cellular concentration of 0.4 g L^{-1} and productivity of 0.008 g L^{-1} h^{-1}, values lower than what was obtained for the medium used in this study, which demonstrates that the strain had a good suitability when it used papaya waste as substrate, as well as the good nutritional source that the papaya waste is.

Analyzing the substrate curves (Figure 2), it was

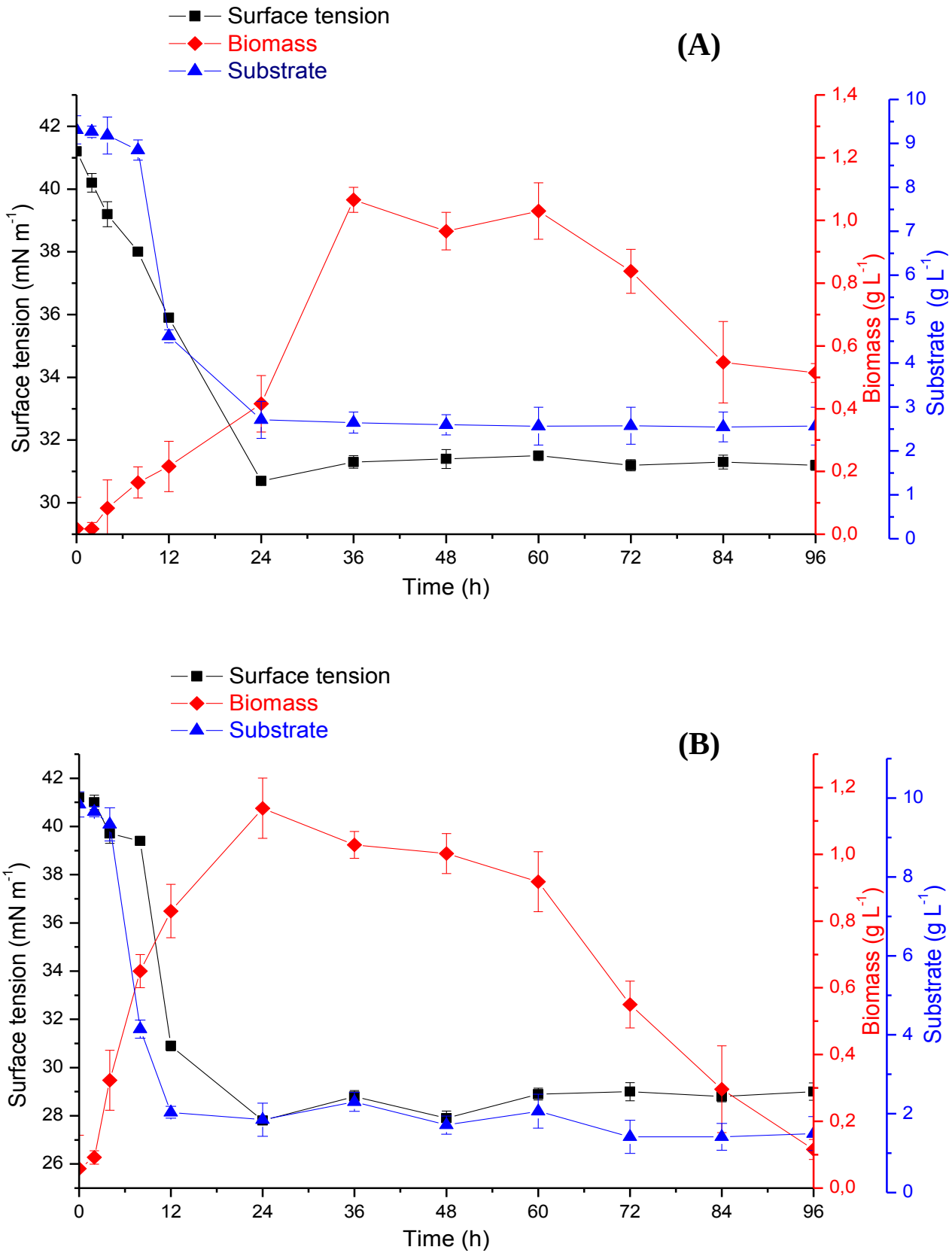

Figure 2. Biomass concentration, substrate consumption and surface tension along 96 h of culture using the aqueous extract prepared from papaya (*Carica papaya* L.) waste as substrate without pH correction (A) and with the pH adjusted to 6.8 (B).

observed that the highest consumption occurred during the exponential phase of microbial growth, as expected, because at this stage, in addition to consuming the substrate for cell maintenance, the microorganism also uses it for development. It was also noted that the consumption was high, with almost substrate exhaustion which may have caused a series of processes that affect the microorganism survival. These processes include: induction of chemotaxis and motility, degradative enzymes synthesis, development of genetic competence, production of peptidic antibiotics and sporulation (Marahiel et al., 1993).

The measurements of surface tension over time is an indirect method to monitor the production of biosurfactants and are therefore of great importance in the study of these bioproducts. As the microorganism grows, it synthesizes the biosurfactant and this metabolite is excreted in the metabolic broth, reducing the surface tension (Lima et al., 2016). Analyzing the behavior of the surface tension for the curve with no pH correction (Figure 2A), it was observed that there was a decrease from 41.2 to 30.7 mN m^{-1}, which means a reduction of approximately 25.5% in 24 h of culture. When the behavior of this variable was observed for the curve with pH correction (Figure 2B), a decrease of 41.2 to 27.8 mN m^{-1} was noted, which means a reduction of about 32.5% in the same 24 h of culture. These values indicate favorable results for the substrate used in the present work since the literature indicates that effective biosurfactants should reduce the surface tension of the medium by at least 20% (Ehrhardt, 2015).

A study by Rocha (2007) using a *B. subtilis* (LAMI007) strain and nutrient broth and diluted cashew juice (varying concentrations of 10 to 50%) as substrates showed no biosurfactant was produced; once biosurfactant production did not occur, there will not be cellular growth and alteration of the surface tension during the 72 h of fermentation, indicating that the proposed medium need supplementation in order to form the bioproduct. In the present study the papaya waste was homogenized at a concentration of 25% (m/v), and cellular growth and reduction of the surface tension occurred, that is, the medium offered sufficient nutrients for the formation of the bioproduct.

Rocha (2007) also carried out the supplementation of the diluted cashew juice with nitrogen sources and verified that there was a reduction in surface tension of about 11.94% after 48 h of cultivation, a lower value and in a longer time than that obtained in the present work.

The study conducted by Ehrhardt (2015) using a strain of *B. subtilis* and pineapple wastes as substrate in the biosurfactant synthesis showed a decrease in surface tension from 64.54 to 48.25 mN m^{-1} after 24 h, which means a reduction of about 25.2%, a value similar to that found in the present study using the papaya waste as substrate without pH correction. However, this value was lower than that obtained when the papaya waste broth with pH corrected to 6.8 was used, which indicates once again the importance of controlling the pH variable to obtain better yields in the fermentation process.

Emulsification index

The emulsification index is also an indirect measurement of the biosurfactant production and it is of great importance since it provides quick results on the formation of biosurfactant and also allows the evaluation of its stability, regarding the emulsion maintenance. Figure 3, plotted using Origin 8.1 (OriginLab CO., MA, USA), shows the emulsification indexes along 96 h of fermentation of papaya residues with no pH modification (Figure 3A) and with 6.8 pH (Figure 3B). The hydrophobic compounds used for emulsification were soy oil, gasoline and diesel.

Analyzing the results of Figure 3, it was observed that both fermented broths were able to emulsify the three tested hydrophobic compounds after 12 h of fermentation, and the highest emulsification was observed for soy oil, whose values were higher than 50% in all the essays. The good emulsification in soy oil suggests a potential use of the produced biosurfactant in the food industry as emulsifying agents, conferring the formation of desired food consistency and texture, as well as phase dispersion (Banat et al., 2000). The emulsification index in soy oil shown in Figure 3A was around 61% and in Figure 3B was around 66% in 12 h of fermentation. In both cases, the emulsification index was bigger than what was found by Silva et al. (2015), who used glucose and sodium nitrate as carbon and nitrogen sources and obtained emulsification index of 34.5% in the fermentation time of 24 h using sunflower oil as a hydrophobic compound. The highest emulsification indexes were obtained in the exponential phase of growth, as expected, because it was in this stage that the most pronounced reduction of the surface tension was observed, indicating a higher biosurfactant production.

The high emulsification index obtained by the soy oil suggests a higher affinity of the biosurfactant produced in the present work by the functional groups found in this oil that is basically formed by fatty acids of 16 and 18 carbons, as reported by Fonseca and Gutierrez (1974). Gasoline and diesel are made up of mixtures of hydrocarbons ranging from 4 to 12 carbons for gasoline and 8 to 16 carbons for diesel, as described by the National Agency for Petroleum, Natural Gas and Biofuels (2016). Figure 3 also shows that the emulsification index for gasoline was superior to the one found for diesel in all tests even though both were formed by a mixture of hydrocarbons. The difference in the emulsification behavior of diesel and gasoline may suggest a better emulsification of short chain hydrocarbons regarding the biosurfactant produced in this paper, as already reported by Barros et al. (2008), but other studies are required for

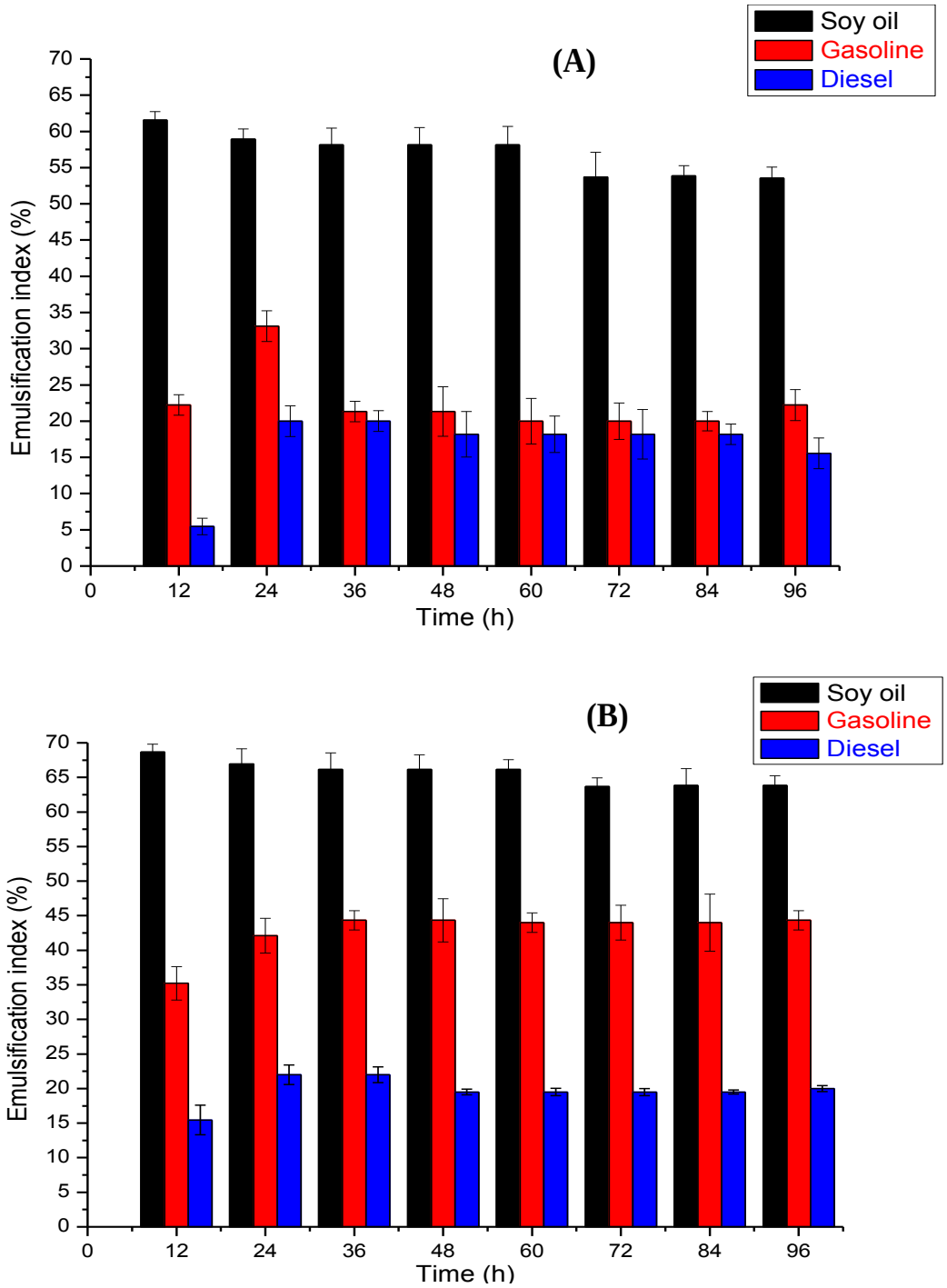

Figure 3. Emulsification indexes of the supernatants along 96 h of fermentation using soy oil, gasoline and diesel as hydrophobic compounds for the papaya wastes without pH correction (A) and with pH correction to 6.8 (B).

this analysis.

Critical micelle concentration

An important distinction to be made is between an effective biosurfactant and an efficient biosurfactant. According to Oliveira et al. (2013), effectiveness is measured by the minimum value to which the surface tension can be reduced, whereas efficiency is measured by the biosurfactant concentration required to produce a significant reduction in the surface tension of water. The

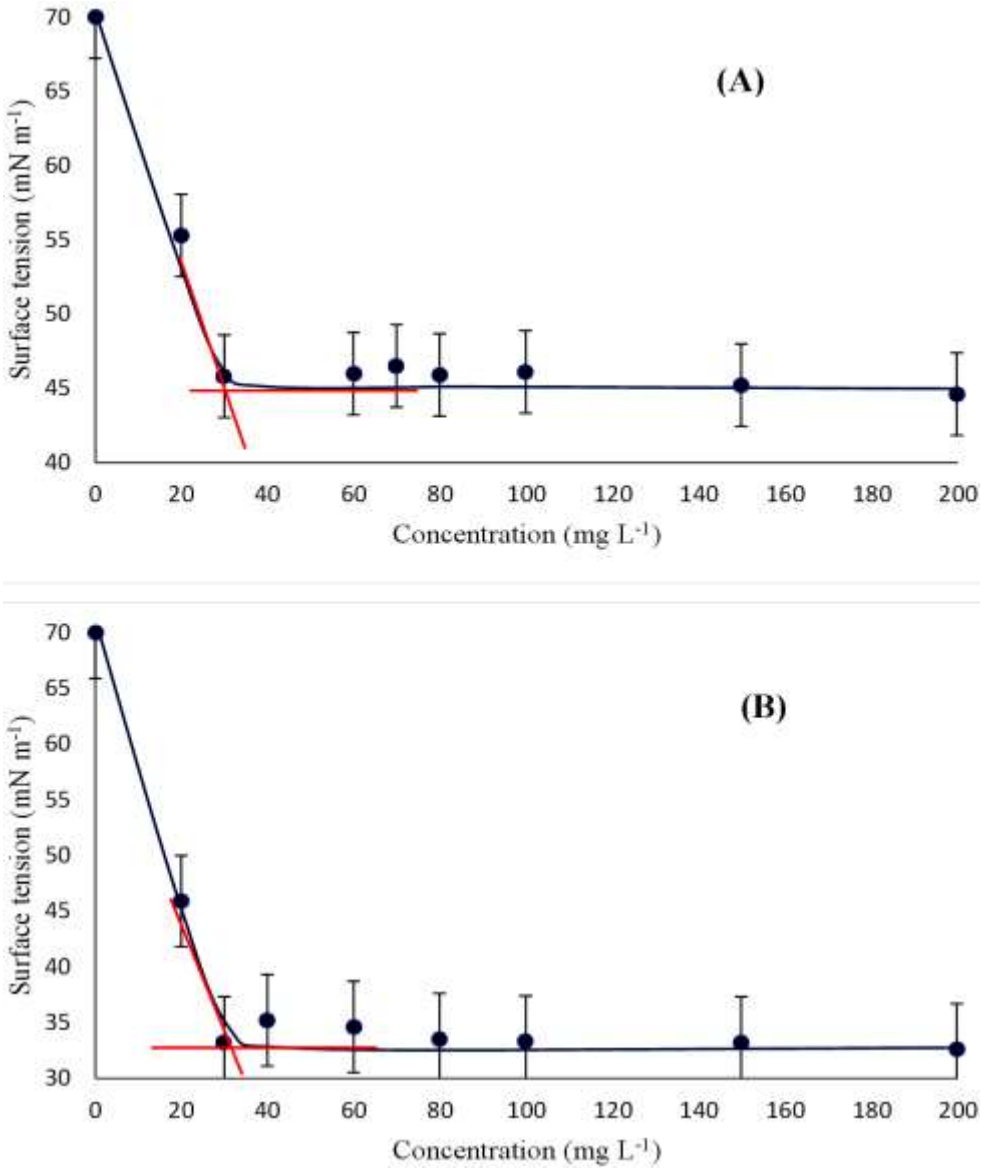

Figure 4. Critical micelle concentration for the fermented medium without pH correction (A) and with pH correction to 6.8 (B).

latter can be determined from the critical micelle concentration (CMC) of the biosurfactant.

CMC is the amphiphilic component concentration in solution at which the micelles formation is initiated. At low biosurfactant concentrations in the fluid, the tendency of the molecules is to agglomerate on the surface. However, the higher the concentration of these compounds on the surface, the greater the tendency to form micelles. At a maximum concentration, the surface will be saturated with biosurfactant to the point that the addition of more biosurfactant will characterize the formation of this molecular aggregate. The lower the CMC, the better the efficiency of the biosurfactant whose concentration values range from 1 to 200 mg L^{-1} (Costa, 2005; Ferreira, 2016). It is important for several biosurfactant applications to establish their CMC, because above this concentration no further effects are observed in the surface activity. Figure 4, plotted using Excel 2016 (Microsoft® CO., WA, USA), shows the critical micelle concentration of the broths without pH correction (Figure 4A) and with 6.8 pH (Figure 4B), using the cell-free fermented broth after 24 h of culture.

Analyzing Figure 4, it is noted that the critical micelle concentration for with and without pH correction corresponded to approximately 35 mg L^{-1}. However, it can be seen in Figure 4A that the CMC is given by the point whose surface tension corresponds to 45.1 mN m^{-1} and in Figure 4B the CMC is obtained at the point where

the surface tension corresponds to 33.3 mN m^{-1}. Although the biosurfactant concentrations obtained were the same (same efficiency), the biosurfactant produced using the substrate with pH correction to 6.8 was more effective in reducing surface tension, which enforces the importance of the substrate and pH variables in the bioprocess.

Reis et al. (2004) investigated biosurfactant production by *B. subtilis* ATCC 6633 using commercial sugar, sugarcane juice, mannitol and soy oil. The commercial sugar had a greater reduction of the surface tension and CMC of 78.6 mg L^{-1} in 48 h of culture, that is, a higher concentration and a longer fermentation time than what was achieved in the present study. Ferreira (2016) used a biosurfactant produced by *B. subtilis* using algaroba (*Prosopis juliflora*) as a substrate for advanced oil recovery and obtained CMC value of 50 mg L^{-1}, also higher than what was obtained in the present study.

Oliveira et al. (2013) obtained CMC from cell-free fermented broths using different initial concentrations of clarified cashew apple juice as substrate and found values between 10 and 65 mg L^{-1}. 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, 35 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.

Conclusion

Papaya (*C. papaya* L.) waste was effective as a substrate source in biosurfactant production by *B. subtilis* UFPEDA 86, since they caused a significant reduction in the surface tension of the medium. In addition, cell growth occurred quickly, which demonstrated the adaptability of the microorganism to this alternative substrate. By correcting the pH of the culture medium to 6.8, a better adaptation of the microorganism and, consequently, a greater reduction of the surface tension was observed, which shows that the pH variable is of great importance regarding the yield of this bioprocess. The results suggest that papaya (*C. papaya* L.) waste can be a valuable substrate for biosurfactant production by *B. subtilis* and can also be an opportunity to reduce the environmental pollution caused by its disposal from the agro-industrial processing. In addition, both the waste producer and the biosurfactant producer will benefit, since the waste will have added value however they will not be as high as that of the conventional substrates.

Therefore, the use of low cost waste to produce a valuable bio-product provides a way to seek technological innovations by reducing costs and increasing profits, which is the main goal of efficient and accurate agricultural management.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank CAPES for the financial assistance, Brasfrut® for the donation of waste, the Federal University of Pernambuco for the donation of the strain and the Federal University of Bahia for the laboratory facilities.

REFERENCES

Agência Nacional do Petróleo, Gás Natural e Biocombustíveis - ANP (2016). Combustíveis líquidos. (Available at http://www.anp.gov.br/wwwanp/petroleo-e-derivados2/combustiveis). Access in Nov. 10, 2017.

Banat IM, Makkar RS, Cameotra SS (2000). Potencial Commercial Applications of Microbial Surfactants. Applied microbiology and biotechnology 53:495-508.

Barros FFC, Quadros CP, Júnior MRM, Pastore GM (2007). Surfactina: propriedades químicas, tecnológicas e funcionais para aplicações em alimentos. Química Nova 30(2):409-414.

Barros FFC, Quadros CP, Pastore GM (2008). Propriedades emulsificantes e estabilidade do biossurfactante produzido por *Bacillus subtilis* em manipueira. Ciência e Tecnologia de Alimentos 28(4):979-985.

Bugay C (2009). Biossurfactantes produzidos por *Bacillus* sp.: Estudos de produção e caracterização. Dissertation, Federal University of Paraná, Curitiba.

Cold Spring Harbor Protocols (CSH) (2010). Luria-Bertani (LB) broth (10%) (Available at http://cshprotocols.cshlp.org/content/2010/7/pdb.rec12257.full?sid=77d7e4c5-9a4d-4050-8961-65adf437a5d5). Access in Sep. 09, 2017.

Cooper DG, Goldenberg BG (1987). Surface-active agents from two *Bacillus* species Applied and environmental microbiology 53(2):224-229.

Costa GAN (2005). Produção biotecnológica de surfactante de *Bacillus subtilis* em resíduo agroindustrial, caracterização e aplicações. Dissertation, Campinas State University, Campinas.

Ehrhardt DD (2015). Produção de biossurfactantes por Bacillus subtilis utilizando do processamento do abacaxi como substrato. Dissertation, Campinas State University, Campinas.

Ferreira GFD (2016). Recuperação avançada de petróleo utilizando sistemas microemulsionados e biossurfactante. Dissertation, Federal University of Bahia, Salvador.

Fonseca H, Gutierrez LE (1974). Composição em ácidos graxos de óleos vegetais e gorduras animais. Anais da Escola Superior de Agricultura Luiz de Queiroz 31:485-490.

Gallert C, Winter J (2002). Solid and liquid residues as raw materials for biotechnology. Naturwissenschaften 89:483-496.

Geys R, Soetaert W, Van Bogaert I (2014). Biotechnological opportunities in biosurfactant production. Current opinion in biotechnology 30:66-72.

Ghojavand H, Vahabzadeh F, Roayaei E, Shahraki AK (2008). Production and properties of a biosurfactant obtained from a member of the *Bacillus subtilis* group (PTCC 1696). Journal of Colloid and Interface Science 324:172-176.

Gondim JAM, Moura MFV, Dantas AS, Medeiros RLS, Santos KM (2005). Composição centesimal e de minerais em cascas de frutas. Ciência e Tecnologia de Alimentos 25(4):825-827.

Gudina EJ, Teixeira JA, Rodrigues LR (2010). Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei*. Colloids Surf. B: Biointerfaces 76: 298-304.

Gudiña EJ, Rangarajan V, Sen R, Rodrigues LR (2013). Potential therapeutic applications of biosurfactants. Trends in pharmacological sciences 34(12): 667-675.

Kuyukina MS, Ivshina IB, Philp JC, Christofi N, Dunbar SA, Ritchkova MI (2001). Recovery of Rhodococcus biosurfactants using methyl tertiary-butyl ether extraction. Journal of Microbiological Methods 46(2):149-156.

Lima JMS, Pereira JO, Batista IH, Neto PQC, Santo JC, Araújo SP, Pantoja MC, Mota AJ, Azevedo JL (2016). Potential biosurfactant producing endophytic and epiphytic fungi, isolated from macrophytes in the Negro River in Manaus, Amazonas, Brazil. African Journal of Biotechnology 15(24):1217-1223.

Lima TA, Rinaldi MM, Ascheri DPR (2008). Características físico-químicas e composição centesimal de cascas e sementes de mamão. In. VI Seminário de Iniciação Científica e III Jornada de Pesquisa e Pós-Graduação. Anápolis: Universidade Estadual de Goiás.

Lobato AKC, Almeida AF, Bezerra MS, Junior LMB, Santos LCL, Macedo GR (2013). Biosurfactant production from industrial residues using microorganisms isolated from oil wells. International Review of Chemical Engineering 5(4):310-316.

Makkar RS, Cameotra SS (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. Applied microbiology and biotechnology 58:428-434.

Marahiel MA, Nakano MM, Zuber P (1993). Regulation of Peptide Antibiotic Production in *Bacillus*. Molecular Microbiology 7(5):631-636.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31:426.

Mukherjee AK, Das LK (2005). Correlation between diverse cyclic lipopeptides production and regulation of growth and substrate utilization by *Bacillus subtilis* strains in a particular habitat. FEMS Microbiology Ecology 54:479-489.

Nitschke M, Ferraz C, Pastore GM (2004). Selection of microorganisms for biosurfactant production using agroindustrial wastes. Brazilian Journal of Microbiology 35:81-85.

Oliveira DWF, França IWL, Félix AKN, Martins JJL, Giro MEA, Melo VMM, Gonçalves LRB (2013). Kinetic study of biosurfactant production by *Bacillus subtilis* LAMI005 grown in clarified cashew apple juice. Colloids and Surfaces B: Biointerfaces 101:34-43.

Reis FA, Sérvulo EF, De França FP (2004). Lipopeptide surfactant production by *Bacillus subtilis* grown on low-cost raw materials. Applied biochemistry and biotechnology 113:899-912.

Rocha MVP (2007). Produção de biossurfactantes por fermentação submersa usando substrato não convencional. Dissertation, Federal University of Ceará, Fortaleza.

Rocha PM (2017). Produção de surfactina por *Bacillus subtilis* UFPEDA 438 utilizando melaço de cana como substrato. Dissertation, Federal University of Rio Grande do Norte, Natal.

Santa Anna LM, Sebastian GV, Menezes EP, Alves TLM, Santos AS, Pereira Jr. N, Freire DMG (2002). Production of Biosurfactants from *Pseudomonas aeruginosa* PA1 Isolated in Oil Environments. Brazilian Journal of Chemical Engineering 19:159-166.

Schmidell W, Lima UA, Aquarone E, Borzani W (2001). Biotecnologia industrial 2. São Paulo, SP: Edgard Blucher. P 541.

Silva RKP, Santos SFM, Almeida AF, Dias PVS, Costa MHJ (2015). Avaliação do crescimento e produção de biossurfactantes por *Bacillus subtilis* e *Bacillus pumilus*. In: I Cong. Nac. Eng. Pet, GN, Bioc. Campina Grande: Univ. Fed. Camp. Grande.

Singh A, Van Hamme JD, Ward OP (2007). Surfactants in microbiology and biotechnology: Part 2. Application aspects. Biotechnology Advances 25:99-121.

Singh PB, Sharma S, Saini HS, Chadha BS (2009). Biosurfactant production by Pseudomonas sp. and its role in aqueous phase partitioning and biodegradation of chlorpyrifos. Letters in Applied Microbiology 49:378-383.

Souza O, Schulz MA, Fischer GAA, Wagner TM, Sellin N (2012). Energia alternativa de biomassa: bioetanol a partir da casca e da polpa de banana. Revista Brasileira de Engenharia Agricola e Ambiental-Agriambi 16:915-921.

Tríboli EPDR (1989). Métodos analíticos para o acompanhamento da fermentação alcoólica. 52f. Apostille – Laboratory of Biochemical and Food Engineering, Mauá Engineering School, Mauá Institute of Technology, São Caetano do Sul.

Tuleva B, Christova N, Cohen R.; Antono D, Todorov Y, Stoineva I (2009). Isolation and characterization of tetraester biosurfactants from a soil strain *Micrococcu luteus* BN56. Process Biochemistry 44:135-141.

Ying G-G (2006). Fate, behavior and effects of surfactants and their degradation products in the environment. Environment international 32:417-431.