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ORIGINAL PAPER

Production of biosurfactant by *Bacillus subtilis* LB5a on a pilot scale using cassava wastewater as substrate

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Abstract The main characteristic of biosurfactants is their property of reducing the superficial and interfacial tension between two immiscible liquids of different polarities. The main obstacle to the application of biosurfactants is the high production costs, the use of alternative substrates being indicated to solve this problem. This work report the production of biosurfactant by *Bacillus subtilis* LB5a on a pilot scale using cassava wastewater as the substrate, and the study of the parameters related to its production. The cassava wastewater was heated, centrifuged and poured into a 40-liter batch pilot bioreactor adapted for simultaneous foam collection during the fermentative process. The temperature was maintained at 35 °C, agitation at 150 rpm and aeration 0.38 vvm during the first 12 h, and 0.63 vvm for the rest of the process. Samples of liquid fermentate were collected at regular intervals for the analysis of total carbohydrates, reducing sugars, pH, CFU/mL count and superficial tension. The foam was centrifuged and the biosurfactant purified. The kinetic data of the process showed that both the microbial population, which reached a maximum after about 24 h, and the foam production of 10.6 L, peaked between 24 and 36 h, coinciding with the greatest production of biosurfactant. The yield of semi-purified surfactant in the foam was 2.4 g/L. The superficial tension of the medium was reduced from 51 to 27 mN/m and the critical micellar concentration was 11 mg/L, which, in principle, characterizes it as a good tensoactive agent. As a function of its composition and productivity, cassava wastewater was identified as a good substrate for the production of the biosurfactant.

Keywords Cassava wastewater · *Bacillus subtilis* · Biosurfactant · Lipopeptides · Pilot scale

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Introduction

Biosurfactants are produced by a great variety of microorganisms, such as bacteria, yeast and fungi [20, 33]. However, bacteria produce the majority of these compounds [16, 20, 28]. Strains of *Bacillus subtilis* are well known for their ability in producing lipopeptides with high surface activity [9, 12, 22, 41, 45] and, amongst these, surfactin, one of the most powerful biosurfactants known, can occur in several isoforms [23–25, 35, 39, 40]. Surfactin reduces the superficial tension of water-solutions from 72 to 27 mN/m, and the interfacial tension to <1.0 mN/m [5, 9, 25, 35, 40]. In addition to surfactin, other surfactant lipopeptides produced by *Bacillus* sp are also known [1, 13, 27, 28].

Although biosurfactants show potential applications in diverse fields, such as the recovery of oil (MEOR), bioremediation, health and food processing, the production costs are still very high [3, 15, 34], and they must also compete with the synthetic surfactants in other aspects such as functionality and production volume. In applications such as the secondary recovery of oil (MEOR), which require elevated amounts of surfactants, they are economically incompatible [29]. However, in products with high added value their use is possible, considering that only small amounts are required, and therefore this kind of use could absorb the high price of the product [15, 51].

A reduction in costs is an important goal to make them economically viable. The use of industrial residues as the medium is a practicable strategy; since the raw material represents about 30% of the total cost in a biotechnological process [7]. The use of agro industrial residues with high levels of carbon sources is indicated as a successful alternative in the production of biosurfactants [29]. Solid potato residue [18, 49] and hydrolyzed peat [45] have been reported already for this purpose. Nitschke et al. [34] tested cassava wastewater, whey and molasses as substrates for biosurfactant- producer *B. subtilis* strains, and obtained better results with cassava wastewater than with the synthetic medium used by Sheppard and Cooper [44].

Cassava wastewater originates from the pressing of cassava roots and is considered to be a harmful residue to the environment due to its organic material load and high levels of cyanide [8, 37]. The production of surfactin in cassava wastewater medium has been described well in the literature [4, 34–36, 38] and the present work represents a stage in the industrial implantation for the production of these compounds. In the earlier works, the biosurfactant recovered from the fermentation in cassava wastewater presented great surface activity. This property was evidenced by the reduction in superficial tension from 50 to 26–27 mN/m and by the small values for the critical micelle concentration (CMC), as well as presenting good heat stability, pH stability and emulsification capacity, suggesting good potential for commercial applications [4, 36].

Some workers have reported the scale-up of the production, indicating a good field for its exploration [3, 15, 32, 42, 50]. The production of biosurfactant by *B. subtilis* on a pilot scale using solid-state fermentation indicated that the packed column bioreactor could become an acceptable system for its industrial production [50]. An experiment for the pilot production of rhamnolipid by *Pseudomonas aeruginosa* was also carried out [42]. However, no work indicating the use of an alternative substrate to the synthetic medium was found. Recovery from the foam has also been described as a method of recovery of the surfactin from the culture medium [11, 53]. The present study was focused on the production of biosurfactant in a pilot bioreactor using cassava wastewater as the culture medium.

Materials and methods

Preparation and characterization of the substrate

Cassava wastewater was collected from a cassava flour factory (Plaza LTDA, Santa Maria da Serra, SP, Brazil) and transported to the place of processing at room temperature. It was homogenized, boiled, cooled, centrifuged at 3,500*g* for 10 min in a pilot-scale basket centrifuge and stored frozen until used. A volume of 40 L of the previously treated cassava wastewater was poured into the pilot bioreactor Pilot New Brunswick 8000 MP 80 (New Brunswick Scientific, Edison, NJ, USA), sterilized at 121 °C for 20 min in the bioreactor and then cooled to the incubation temperature.

The substrate was characterized by analyses for total nitrogen [2], total [10] and reducing [46] carbohydrates, the mineral fraction (P, K, Ca, Mg, S, Al, B, Cu, Fe, Mn, Zn, Cd, Cr, Ni and Pb) [17], ammonia, nitrate [6] and pH. All experiments were carried out with the same substrate.

Microorganism and inoculum preparation

A strain of *B. subtilis*, previously isolated and identified as LB5a, pertaining to the culture collection of the Bioflavors Laboratory of DCA/FEA/Unicamp [34], was used. A loop of culture growth taken from a Petri dish was transferred to a conical flask containing 150 mL of nutrient broth and maintained at 30 °C for 12 h in a rotary shaker bath at a speed of 150 rpm. The medium used for experiments contained (g/L distilled water): beef extact 1.0, yeast extract 2.0, peptone 5.0 and sodium chloride 5.0. The culture was then homogenized and 5 mL aliquots transferred to 24 flasks, each containing 100 mL of nutrient broth, followed by incubation at 30 °C in a rotary shaker bath with agitation at 150 rpm for 8 h. The inoculums were standardized by measuring the optical density at $\lambda = 610$ nm. Thus, considering the standard curve for cell concentration versus optical density, a number of flasks sufficient for the culture to reach the initial concentration of 2×10^7 CFU/mL were taken. Sterile nutrient broth, prepared under the same conditions, was used as the blank, and a parallel standard count was carried out.

Fermentative process/foam recovery system

The inoculum, using an amount calculated previously in the standardization phase, was added to the sterile cassava wastewater. Fermentation was carried out according to the following parameters: temperature 35 °C, agitation 150 rpm and 15 L/h of aeration (0.38 vvm) during the first 12 h, followed by 25 L/h (0.63 vvm) for the rest of the process. A sterile silicon tube (internal diameter of 20 mm) was connected to the top of bioreactor to recover the biosurfactant by withdrawing the foam produced during the process [11, 22] (Fig. 1). Samples of the medium were collected at regular, approximately 12 h, intervals, as well as all the foam accumulating in the collecting flask, and stored for future analysis. The pH and temperature were also verified. The following analyses were carried out of the medium samples collected: viable cell count [36], surface tension [36], total carbohydrates [10] and reducing sugars [46].

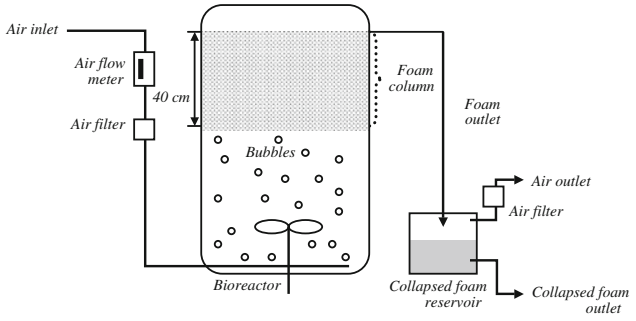

Fig. 1 Apparatus scheme used in the experiments—growth of *Bacillus subtilis* LB5a and recovery of the foam

The foam was collapsed at room temperature and its volume and pH measured. It was then submitted to the purification process to obtain the surfactant production data. A foam sample of approximately 15 mL was then removed to determine the surface tension and the critical micelle concentration. The concentration of biosurfactant in the collapsed foam was determined by rate of weight of the purified biosurfactant and the volum of collapsed foam.

Extraction and purification of the surfactant

The liquefied foam collected throughout the fermentative process was centrifuged at $12.7 \times 10^3 g$ for 20 min to remove the cells. Its pH was adjusted to 2 using a 2 N HCl solution and maintained at rest for 12 h for decantation. The liquid phase was then centrifuged at $12.7 \times 10^3 g$ for 20 min and the precipitate neutralized using a 1 N NaOH solution before drying at 50 °C [30, 31]. After drying, the solid residue was weighed, triturated in a mortar, gimbaled in a solution of chloroform/methanol 65:15 and filtered through Whatman no°1 filter paper. After filtering, the permeate was dried again at 50 °C. The solid residue was triturated, weighed and stored frozen for subsequent analysis.

Determination of the critical micelle concentration

The critical micelle concentration (CMC) was determined by measuring the surface tension of serial dilutions of a 1 mg/mL solution of the semi-purified biosurfactant according to Sheppard et al. [45] in Krüss GmbH K-12 processor tensiometer (Hamburg, Germany).

Results and discussion

Cassava composition

The composition of the cassava wastewater is shown in Table 1. The cassava wastewater presented a good level of carbohydrates, characterizing it as a good substrate for the development of microorganisms, as well as for the production of biosurfactants [26, 43]. The presence of high levels of several important micronutrients for microbial development makes it an important and promising substrate, since it does not require supplementation.

The Mn^{2+} concentration was 1.6 mg/L, a value very close to that of the supplemented synthetic medium used by Wei and Chu [51] of 1.1 mg/L, which, according to the authors, provided a significant increase in cell growth and the production of surfactin. The Fe^{2+} concentration was also considered to be important, since Wei and Chu [52] and Cooper et al. [9] used 0.2 mg/L of Fe^{2+} with good results. The Zn^{2+} and NH^{4+} ions have also been considered important to increase the production of the biosurfactants [19]. Other nutrients that affect this production are phosphate and other sources of nitrogen [26].

Although the nitrogen concentration has an important role in the production of biosurfactants [12, 26], supplementation of the medium was discouraged by the results found in previous studies. The supplementation of cassava wastewater with yeast extract, peptone, urea, ammonium nitrate, steep liquor (sub-product of the wet maceration of

Table 1 Physicochemical composition of cassava wastewater

Components (Units)	Concentration	Components (Units)	Concentration
Total carbohydrates (g/L)	36.2	Iron (mg/L)	4.2
Reducing sugars (g/L)	13.9	Manganese (mg/L)	1.6
Total nitrogen (g/L)	1.92	Zinc (mg/L)	1.4
Phosphorous (mg/L)	246	Cadmium (mg/L)	<0.01
Potassium (mg/L)	894	Chromium (mg/L)	<0.01
Calcium (mg/L)	212	Nickel (mg/L)	<0.01
Magnesium (mg/L)	288	Lead (mg/L)	<0.01
Sulfur (mg/L)	150	Ammonia [NH_4^+] (mg/L)	131
Aluminium (mg/L)	148	Nitrate [NO_3^-] (mg/L)	16.2
Boron (mg/L)	4.0	pH	5.4
Copper (mg/L)	0.3		

corn) and whey, none of which resulted in a significant increase in the amount of biosurfactant produced, and, in addition, made recovery more difficult (data not published). A further analysis of the cassava wastewater composition shows a prevalence of NH_4^+ ions instead of NO_3^- ions. Previous studies showed that *B. subtilis* preferred organic nitrogen, ammonium and nitrate, respectively, in the production of tensoactive compounds [12]. Detectable amounts of heavy metals (Ni, Cd, Pb and Cr) were not found by the analytical methods used.

All the characteristics described above demonstrate the potential use of the cassava wastewater without supplementation, showing excellent results concerning the production of biosurfactants.

Process kinetics

The kinetics were evaluated as a function of the concentrations of total and reducing sugars, pH, viable CFU count, surface tension of the medium, volume of liquefied foam recovered, production of semi purified biosurfactant and concentration of surfactant per liter of liquefied foam. Nine repetitions for the fermentation process were carried out. Each analysis of total and reducing sugars, pH, viable CFU count and surface tension of the medium were carried out in duplicate.

When the total and reducing sugar concentrations and viable cell counts were compared, it could be seen that the carbohydrates were the preferred carbon sources of the microorganism, and thus their concentrations fell as the *B. subtilis* population grew. This is in accordance with Sandrin et al. [43], who affirmed that glucose, fructose and sucrose were the best carbon sources for surfactin synthesis. The concentration of total sugars fell by about 76% (from 36.2 to 8.7 g/L) in the first 36 h. This significant reduction in the sugars coincided with the log phase and the beginning of the stationary phase. A comparison of the sugar consumption revealed a strong relation with biosurfactant production. From start of process until 36 h occurred increased both the consumption of sugars and of the biosurfactant production, after this period both decrease (Fig. 2). Kim et al. [22] reported an almost direct relationship between biosurfactant production, cell growth and glucose consumption when using a synthetic medium. Despite the high consumption, the reducing sugar concentration increased during the first 12 h of the process (Fig. 2). This behavior was probably a consequence of the action of amylolitics enzymes produced by the *Bacillus*, which is known as a good producer of these enzymes [21, 47]. α-amylases present in the medium must have hydrolyzed the starch, liberating reducing sugars (glucose) in amounts greater than those consumed by the culture. With the increase in consumption of these sugars in the following hours and the exhaustion of the more complex sugars, the concentration stopped increasing and started decreasing, reaching a minimum value of 4 g/L after 48 h. This hypothesis agrees with the studies of Thompsom et al. [48], in which, the expression of *B. subtilis* α-amylase allowed for the use of potato residue as a starch-rich medium for biosurfactant production, and of Nitschcke [36], who showed an increase in the concentration of the reducing sugars, glucose, fructose and maltose, when the strain LB5a was cultivated in cassava

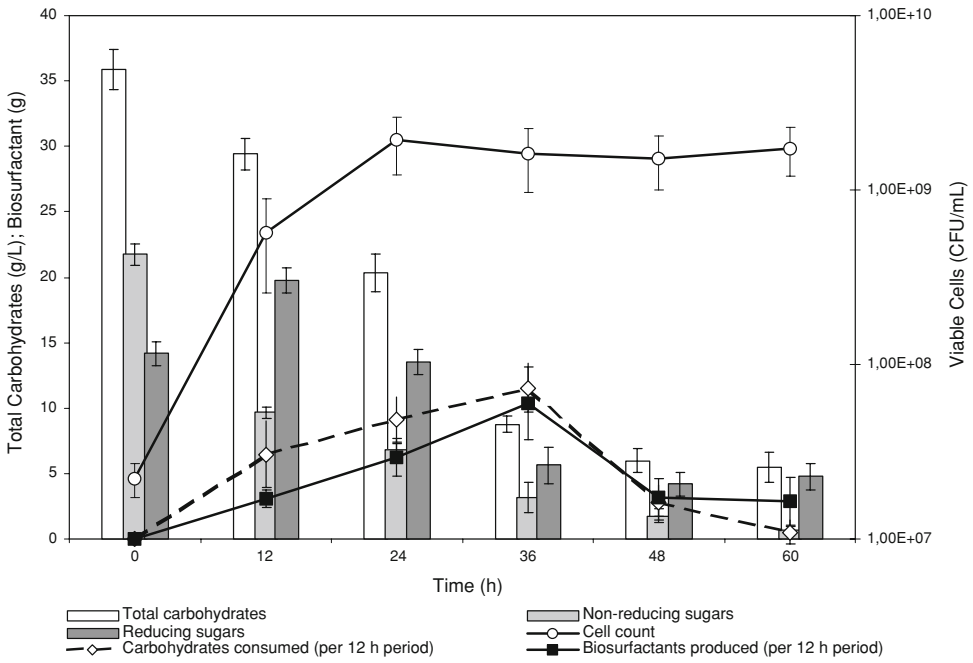

Fig. 2 Culture kinetics: cell growth, biosurfactant production and carbohydrate consumption

wastewater. This increase was attributed to the hydrolysis of the sucrose and starch present in the medium.

An increase in the amount of foam collected was associated with a greater production of biosurfactant, which occurs basically in the log phase and at the beginning of the stationary phase [9, 22, 23, 49]. This production was made evident in the first 12 h by the accentuated fall in surface tension from 52 to 33 mN/m (Fig. 3). However, the continuous pull of the tensoactive compounds maintained the concentration stable and did not allow any great variation in the surface tension between 12 and 24 h. After this period, the population reached the stationary phase and, consequently, the production of tensoactive compounds was reduced, and at the same time the continuous withdrawal of the surfactant made its concentration in the medium fall, resulting in an increase in tension at the end of the process. This information corroborated the data of Razafindralambo et al. [41] and Davis et al. [11] referring to the efficiency of foam production as a form of primary recovery of the biosurfactant. During the process, the pH value varied positively from 5.40 to 7.63. However, the majority of this variation was verified between 0 and 36 h, after which it was not significant. Thus, this behavior could be associated with the exponential growth as described by Kim et al. [22] and, consequently, with the synthesis of compounds of interest, since its value became practically steady at the same time as the viable cell count reached the stationary phase and the surface tension increased. When the viable cell count was evaluated, it was possible to identify the exponential growth phase, reaching the maximum count of 2.3×10^9 CFU/mL, after 24 h. A slight reduction in the count was verified in the interval between 24 and 36 h, after which it remained stable. The reduction in the viable cell count during the stationary period seems to be related to the continuous removal of cells into the foam, a phenomenon that occurs when the primary recovery process is via foam production [53]. The slight reduction in the number of viable cells in the last 24 h of the process was directly based on the reduction in the volume of foam recovered, as can be seen in Figs. 2 and 4.

When the foam recovery process was evaluated (Fig. 4), an increasing speed in foam production was observed in the first 36 h. The maximum volume produced was observed in the period between 24 and 36 h, having produced a total of 9.1 L (approximately 0.76 L/h) in this period. The volume then fell to values of about 1.5 L for each 12 h interval. At the end of the fermentation period, approximately 60 h after the start of the process, foam production had already ceased.

The concentration of biosurfactant in the foam was not constant throughout the process. As shown in Fig. 4, the semi-purified biosurfactant concentration in the foam oscillated in the range from 2.5 to 3.0 g/L of foam collected up to 36 h. This concentration then fell to values between 1.5 and 2.0 g/L of foam at the end of the fermentation period, and this reduction can be related to the reduction in the biosurfactant production associated with the recovery process. The joint concentration data corroborated the assumption made from the kinetics of production versus recovery. In studies carried out with cassava wastewater and the same culture (LB5a), Nitschke et al. [35] also found variable concentrations throughout the process, showing similar kinetic profiles.

With respect to fermentation productivity, an average foam volume of 10.6 L was obtained and an average production of 25.7 g of semi purified biosurfactant per batch, giving an average of 2.42 g/L in the foam collected. Considering the initial volume of cassava wastewater, this gives

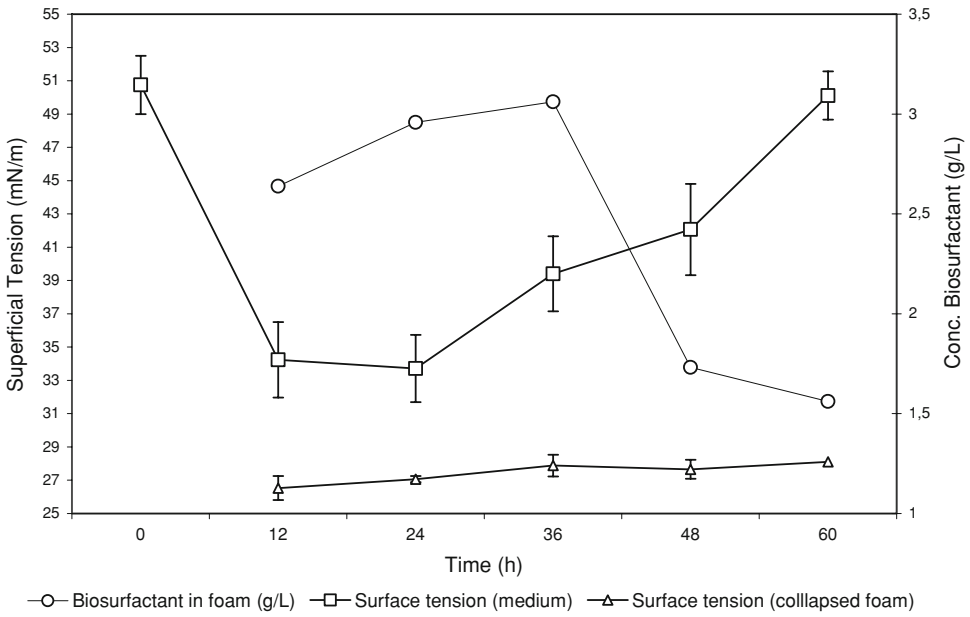

Fig. 3 Surface tension of the medium and collapsed foam recovered throughout the process

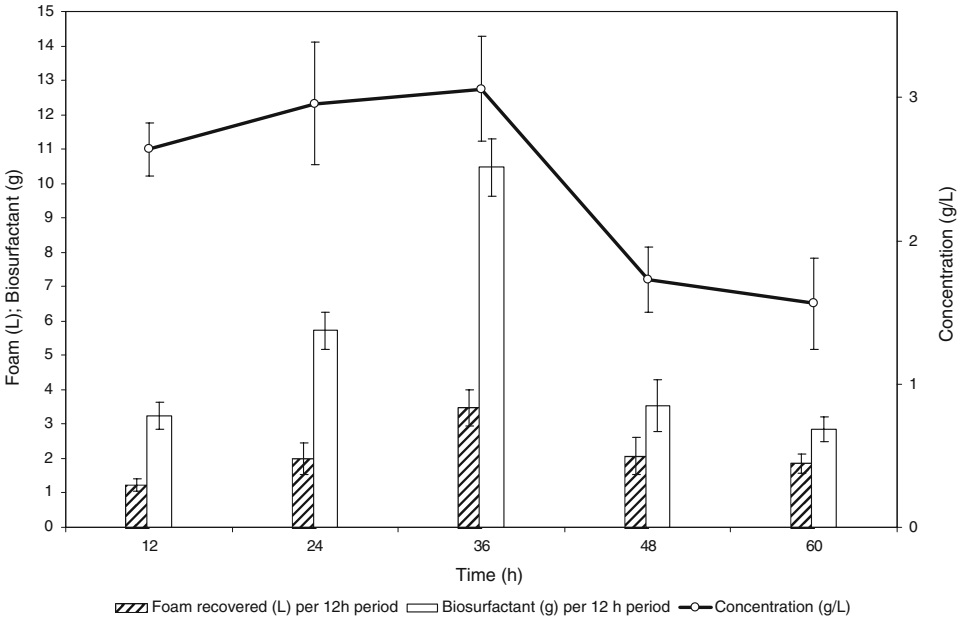

Fig. 4 Kinetics of the production of biosurfactants by Bacillus subtilis LB5a

a yield of 0.64 g of biosurfactant per liter of substrate. A comparison between the values for surface tension of the medium and those of the foam (Fig. 4) demonstrated the efficiency of the process of primary recovery by foam collection, since at all times the foam presented values close to 27 mN/m, while the surface tension of the medium was always above 33 mN/m. When these values for surface tension were compared with the values found in the series of dilutions used to determine the CMC of the culture medium, a significant difference in the amount of surfactant was observed. With about 0.25 g/L of semi-purified surfactant, the surface tension was reduced to 27.5 mN/m, whereas as little as 0.012 g/L was enough to result in a value for surface tension of 32 mN/m.

The CMC of the semi-purified biosurfactant was 11 mg/L. This value is close to those found by, Nitschke [35] 11 mg/L, Sheppard and Mulligan [45] 14 mg/L, Deleu et al. [14] 10 mg/L and described by Peypoux et al. [40] <20 mg/L; and better than those found by Kim et al. [22] 40 mg/L, and Cooper et al. [9] 25 mg/L, characterizing it as a powerful surface active agent. The differences amongst the results are probably due to the medium composition, culture conditions and strain considered. These values are significant, since, in principle, the lower the value of the CMC, the more efficient the compound will be as a surfactant, and therefore the greater its potential for industrial application [5].

Conclusions

Cassava wastewater can be considered to show good potential as a substrate for the production of biosurfactants by *B. subtilis*, due to its contents of nutrients such as carbohydrates, metallic ions, nitrogen sources and others, that make nutritional supplementation unnecessary. The biosurfactants produced in this medium presented high surface activity and low CMC values, characterizing them as good surfactants. For the first time the production of these compounds using agro industrial residues on a pilot scale was demonstrated to be a viable process. The average foam production in each batch was 10.6 L (26.5% of the original volume). After purification, an average of 25.7 g of surfactant was recovered per batch (0.68 g of surfactant/L cassava wastewater). The primary recovery from the foam collected proved to be efficient, as shown by the difference between the surface tension (and concentration of the biosurfactant) of the collapsed foam (~27 mN/m) and that of the culture medium at the end of the process (~50 mN/m). All of these data demonstrate the potential of the process.

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References

1. Ahimou F, Jaccques P, Deleu M (2000) Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. Enzyme Microb Technol 27:749–754. doi:10.1016/S0141-0229(00)00295-7
2. AOAC (1995) Official method of analysis, 16th edn. AOAC International, Arlington
3. Banat IM, Makkar RS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53:495–508. doi:10.1007/s002530051648
4. Barros FFC, Quadros CP, Maróstica MR Jr, Pastore GM (2007) Surfactina: propriedades químicas, tecnológicas e funcionais para aplicação em alimentos. Quim Nova 30:409–414
5. Bognolo G (1999) Biossurfactants as emulsifying agents for hydrocarbons. Colloids Surf 12:41–52

6. Bremmer JM, Keeney DR (1965) Steam-distillation methods for determination of ammonium, nitrate and nitrite. Anal Chim Acta 32:485–495. doi:10.1016/S0003-2670(00)88973-4
7. Cameotra SS, Makkar RS (1998) Synthesis of biosurfactants in extreme conditions. Appl Microbiol Biotechnol 50:520–529. doi:10.1007/s002530051329
8. Cereda MP (2005) Produtos e subprodutos. In: Souza LS, Farias ARN, Mattos PLP, Fukuda WMG (eds) Processamento e utilização da mandioca. Embrapa Mandioca e Fruticultura Tropical, Cruza das Almas
9. Cooper DG, Macdonald CR, Duff SJB, Kosaric N (1981) Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. Appl Environ Microbiol 42:408–412
10. Daniels L, Hanson R, Phyllips JA (1994) Chemical analysis. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington
11. Davis DA, Lynch HC, Varley J (2001) The application of foaming for the recovery of surfactin from *Bacillus subtilis* ATCC 21332 cultures. Enzyme Microb Technol 28:346–354. doi:10.1016/S0141-0229(00)00327-6
12. Davis DA, Lynch HC, Varley J (1999) The production of surfactin in batch culture by *Bacillus subtilis* ATCC 21332 is strongly influenced by the conditions of nitrogen metabolism. Enzyme Microb Technol 25:322–329. doi:10.1016/S0141-0229(99)00048-4
13. Deleu M, Paquot M, Nylander T (2005) Fengycin interaction with lipid monolayers at the air-aqueous interface-implications fopr the effect of fengycin on biological membranes. J Colloid Interface Sci 283:358–365. doi:10.1016/j.jcis.2004.09.036
14. Deleu M, Razafindralambo H, Popineau Y, Jacques P, Thonart P, Paquot M (1999) Interfacial and emulsifying properties of lipopeptides from *Bacillus subtilis*. Colloids Surf A Physicochem Eng Asp 152:3–10. doi:10.1016/S0927-7757(98)00627-X
15. Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potencial. Microbiol Mol Biol Rev 61:47–64
16. Desai JD, Desai AJ (1993) Production of biosurfactants. In: Kosaric N (ed) Biosurfactants: production, properties, applications. Marcel Decker, New York
17. EPA—US Environmental Protection Agency (1994) SW-846 Tests methods for evaluating solid wastes: Physical and chemicam methods. Washington
18. Fox SL, Bala GA (2000) Production of surfactant from *Bacillus subtilis* ATCC 21332 using potato substrates. Bioresour Technol 75:235–240. doi:10.1016/S0960-8524(00)00059-6
19. Gu X, Zheng Z, Yu H, Wang J, Liang F, Liu R (2005) Optimization of medium constituents for a novel lipopeptide production by *Bacillus subtilis* MO-01 by a response surface method. Process Biochem 40:3196–3201. doi:10.1016/j.procbio.2005.02.011
20. Healy MG, Devine CM, Murphy R (1996) Microbial production of biosurfactants. Resour Conserv Recycling 18:41–57. doi:10.1016/S0921-3449(96)01167-6
21. Huang H, Ridgway D, Gu T, Moo-Young M (2004) Enhanced amylase production by *Bacillus subtilis* using a dual exponential feeding strategy. Bioprocess Biosyst Eng 27:63–69. doi:10.1007/s00449-004-0391-z
22. Kim H, Yoon B, Lee C, Suh H, Oh H, Katsuragi T et al (1997) Production and properties of a lipopeptide biosurfactant from *Bacillus subtilis* C9. J Ferment Bioeng 84:41–46. doi:10.1016/S0922-338X(97)82784-5
23. Kluge B, Vater J, Salnikow J, Eckart K (1988) Studies on the biosynthesis of surfactin, a lipopeptide antibiotic from *Bacillus subtilis* ATCC 21332. FEBS Lett 231:107–110. doi:10.1016/0014-5793(88)80712-9
24. Kowall M, Vater J, Kluge B, Stein T, Franke P, Ziessow D (1998) Separation and characterization of surfactin isoforms produced by *Bacillus subtilis* OKB 105. J Colloid Interface Sci 204:1–8. doi:10.1006/jcis.1998.5558
25. Lang S (2002) Biological amphiphiles (microbial surfactantes). Curr Opin Colloid Interface Sci 7:12–20. doi:10.1016/S1359-0294(02)00007-9
26. Lin SC (1996) Biosurfactants: recents advances. J Chem Technol Biotechnol 66:109–120. doi:10.1002/(SICI)1097-4660(199606)66:2<109::AID-JCTB477>3.0.CO;2-2
27. Lin SC, Carswell KS, Sharma MM, Georgiou G (1994) Continuous production of the lipopeptide biosurfactant of Bacillus licheniformis JF-2. Appl Microbiol Biotechnol 41:281–285. doi:10.1007/BF00221219
28. Maier RM (2003) Biosurfactants: evolution and diversity in bacteria. Adv Appl Microbiol 52:101–121. doi:10.1016/S0065-2164(03)01004-9
29. Makkar RS, Cameotra SS (2002) An update on the use of unconventional substrates for biosurfactant production and their new applications. Appl Microbiol Biotechnol 58:428–434. doi:10.1007/s00253-001-0924-1
30. Makkar RS, Cameotra SS (1997) Biosurfactant production by a thermophilic *Bacillus subtilis* strain. J Ind Microbiol Biotechnol 18:37–42. doi:10.1038/sj.jim.2900349
31. Makkar RS, Cameotra SS (1999) Structural characterization of a biosurfactant poduced by Bacillus subtilis at 45 °C. J Surfactants Deterg 2:367–372
32. Mulligan CN, Gibbs BF (1990) Recovery of biosurfactants by ultrafiltration. J Tech Biotechnol 47:23–29
33. Mulligan C (2005) Environmental applications for biosurfactants. Environ Pollut 133:183–198. doi:10.1016/j.envpol.2004.06.009
34. Nitschke M, Ferraz C, Pastore GM (2004) Selection of microorganisms for biosurfactant production using agro industrial wastes. Braz J Microbiol 35:81–85. doi:10.1590/S1517-83822004000100013
35. Nitschke M, Haddad R, Costa GAN, Gilioli R, Meurer EC, Gatti MS et al (2004) Strutural characterization and biological properties of a lipopeptide surfactant produced by *Bacillus subtilis* on cassava wastewater medium. Food Sci Biotechnol 13:591–596
36. Nitschke M, Pastore GM (2006) Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. Bioresour Technol 97:336–341. doi:10.1016/j.biortech.2005.02.044
37. Pantaroto S, Cereda MP (2000) Linamarina e sua decomposição no ambiente. In: Cereda MP (ed) Manejo, uso e tratamento de subprodutos da industrialização da mandioca, vol 4. Fund, Cargill
38. Pastore GM, Santos CFC, Nitschcke M (2003) Processo de Produção de Biosuractante por *Bacillus subtilis*, utilizando resíduo da indústria de mandioca. Br Pat PI 0303853-0.
39. Peypoux F, Bonmatin JM, Labbé H, Das BC, Ptak M, Michel G (1991) Isolation and characterization of a new variant of surfactin, the [Val7] surfactin. Eur J Biochem 202:101–106. doi:10.1111/j.1432-1033.1991.tb16349.x
40. Peypoux F, Bonmatin JM, Wallach J (1999) Recents trends in biochemistry of surfactin. Appl Microbiol Biotechnol 51:553–563. doi:10.1007/s002530051432
41. Razafindralambo H, Paquot M, Baniel A, Popineau Y, Hbid C, Jacques P et al (1996) Foaming properties of surfactin, a lipopeptide biosurfactant from *Bacillus subtilis*. J Am Oil Chem Soc 73:149–151. doi:10.1007/BF02523463
42. Reiling HE, Thanei-Wyss U, Guerra-Santos LH, Hirt R, Käppeli O, Fiechter A (1986) Pilot plant production of rhaminolipid biosurfactant by *Pseudomonas aeruginosa*. Appl Environ Microbiol 51:985–989
43. Sandrin C, Peipoux F, Michel G (1990) Coproduction of surfactin and iturin A lipopeptides with surfactant and antifungal properties by *Bacillus subtilis*. Biotechnol Appl Biochem 12:370–375

44. Sheppard JD, Cooper DG (1991) The response of *Bacillus subtilis* ATCC 21332 to manganese during continuous-phased growth. Appl Microbiol Biotechnol 35:72–76. doi:10.1007/BF00180639
45. Sheppard JD, Mulligan CN (1987) The production of surfactin by *Bacillus subtilis* grown on peat hydrolysate. Appl Microbiol Biotechnol 27:110–116. doi:10.1007/BF00251931
46. Somogyi M (1945) A new reagent for determination of sugars. J Biol Chem 160:61–68
47. Thoma J, Brother C, Spradlin J (1970) Subsite mapping of enzymes. Studies on Bacillus subtilis amylase. Biochemistry 9:1768–1775. doi:10.1021/bi00810a016
48. Thompson DN, Fox SL, Bala GA (2000) Biosurfactants from potato process effluents. Appl Biochem Biotechnol 84:917–929. doi:10.1385/ABAB:84-86:1-9:917
49. Vater J (1986) Lipopeptides, an attractive class of microbial surfactants. Prog Colloid Polym Sci 72:12–18. doi:10.1007/BFb0114473
50. Veenanadig NK, Gowthaman MK, Karath NGK (2000) Scale up for the production of biosurfactant packed column bioreactor. Bioprocess Eng 32:95–99. doi:10.1007/s004490050017
51. Wei Y, Chu I (2002) Mn^{2+} improves surfactin production by *Bacillus subtilis*. Biotechnol Lett 24:479–482. doi:10.1023/A:1014534021276
52. Wei Y, Chu I (2004) Optimizing iron supplement strategies for enhanced surfactin production with *Bacillus subtilis*. Biotechnol Prog 20:979–983. doi:10.1021/bp030051a
53. Yeh M, Wei Y, Chang J (2006) Bioreactorndesign for enhanced carrier-assisted surfactin production with *Bacillus subtilis*. Process Biochem 41:1799–1805. doi:10.1016/j.procbio.2006.03.027