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Evaluation of Cashew Apple Juice for Surfactin Production by *Bacillus subtilis* LAMI008

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Abstract *Bacillus subtilis* LAMI008 strain isolated from the tank of Chlorination at the Wastewater Treatment Plant on Campus do Pici in Federal University of Ceará, Brazil has been screened for surfactin production in mineral medium containing clarified cashew apple juice (MM-CAJC). Results were compared with the ones obtained using mineral medium with glucose PA as carbon source. The influence on growth and surfactin production of culture medium supplementation with yeast extract was also studied. The substrate concentration analysis indicated that *B. subtilis* LAMI008 was able to degrade all carbon sources studied and produce biosurfactant. The highest reduction in surface tension was achieved with the fermentation of MM-CAJC, supplemented with yeast extract, which decreased from 58.95±0.10 to 38.10±0.81 dyn cm^{-1}. The biosurfactant produced was capable of emulsifying kerosene, achieving an emulsification index of 65%. Surfactin concentration of 3.5 mg L^{-1} was obtained when MM-CAJC, supplemented with yeast extract, was used, thus indicating that it is feasible to produce surfactin from clarified cashew apple juice, a renewable and low-cost carbon source.

Keywords Biosurfactants · Raw material · Cashew apple juice · *B. subtilis* LAMI008 · Surfactin · Fermentation

Introduction

Surfactants are surface-active compounds capable of reducing surface and interfacial tension at the interface between liquids, solids, and gases, thereby allowing them to mix or to disperse readily as emulsions in water or other liquids [1, 2]. The enormous market

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demand for surfactants is currently provided by numerous synthetic, mainly petroleum-based, chemical surfactants. However, these compounds are usually toxic to the environment and non-biodegradable. They may bioaccumulate, and their production, processes, and by-products can be environmentally hazardous. Tightening environmental regulations and increasing awareness for the need to protect the ecosystem have effectively resulted in an increasing interest in biosurfactants as possible alternatives to chemical surfactants [3]. Biosurfactants are surfactants produced extracellularly or as part of the cell membrane by bacteria, yeasts, and fungi from various substrates including sugars, oils, alkanes, and wastes [4, 5]. They are amphiphilic compounds with considerable potential in commercial applications within various industries enhancing oil recovery, crude oil drilling lubricants, bioremediation of water-insoluble pollutants, and uses in the health care and food processing industries [6, 7].

Biosurfactants have advantages over their chemical counterparts in biodegradability and effectiveness at extreme of temperature and pH and in having lower toxicity [1]. Nevertheless, currently, they are not widely utilized due to high production costs associated with use of expensive substrates and inefficient product recovery methods [8].

Bacillus subtilis is considered a suitable microorganism for biosurfactant production owing to the absence of pathogenicity, which permits the use of its products in food and pharmaceutical industries [9]. Therefore, discovery of new strains and improvement on culturing methods of *B. subtilis* can provide a safe source of biosurfactants.

Surfactin, one of the most effective cyclic lipopeptides biosurfactants produced for *B. subtilis*, can lower the surface tension of water from 72 to 27 mN m^{-1} and the interfacial tension of the water/*n*-hexadecane system from 43 to 1 mN m^{-1} [8]. The primary structure of surfactin is a cyclic lipopeptide consisting of seven amino acids bonded to the carboxyl and hydroxyl groups of a 14-carbon fatty acid [10]. In addition to its strong surface activity, surfactin also inhibits clot formation, lyses erythrocytes [11], and lyses bacterial spheroplasts and presented antimicrobial activity. Therefore, surfactin has a potential in a variety of commercial applications. However, the high cost and low yield involved in surfactin production appeared to limit its applications [12, 13]. Thus, the economics of biosurfactant production may be significantly impacted through use of inexpensive carbon substrates.

Biosurfactants can be produced in *B. subtilis* cultures using sugars (sucrose, glucose, and lactose), vegetable oils, or starch as carbon sources [8, 13–15]. Alternative source of culture media, as sugar cane, molasses [8, 16], and industrial effluent wastes [7], has been considered to reduce the final cost of the process and obtain higher productivity of biosurfactants.

In the northeast of Brazil, the cashew nut agroindustry has an outstanding role in the economy. Different from the economic value of the nut, the pseudo-fruit or apple from cashew tree is not completely explored. Furthermore, the majority of the cashew apple production spoils in the soil [17–19]. Those facts, together with its rich composition (reducing sugar, fibers, vitamins, and minerals salts), turn cashew apple juice (CAJ) into an interesting and inexpensive (R$ 1.00/kg) culture medium [20].

Cashew is produced in around 32 countries of the world, and the major cashew apple producing countries are Vietnam (8.4 million tons), Nigeria (5 million tons), India (4 million tons), Brazil (1.6 million tons), and Indonesia (1 million tons) [21]. The official estimate for the Brazilian cashew crop 2008/2009 was around 300 thousand of tons [22], which accounts for 11% of the world production and corresponds to more than 6 million tons of cashew apple. Taking into account that the use of agroindustrial residues can contribute for the reduction of production costs, cashew apple appears as an alternative raw

material for biosurfactant production due to its vast availability and high concentration of reducing sugars.

Therefore, the aim of this work was to develop a growth medium using cashew apple as carbon and energy source to cultivate a new isolate of *B. subtilis* LAMI008 and to produce biosurfactant.

Materials and Methods

Microorganism and Culture Media

The strain of *B. subtilis* LAMI008 used in this study was isolated from the tank of chlorination at the Wastewater Treatment Plant on Campus do Pici (WWTP-PICI) in the Federal University of Ceará, Brazil. Its rRNA 16S sequence is deposited in the Genbank with the following access number: EU082292. The strain was maintained on Nutrient agar (Merck, Germany) slants at 4 °C and transferred monthly. The inoculum medium was APGE (consisting of peptone, 5.0 g L^{-1}, glucose 5.0 g L^{-1}, yeast extract 2.5 g L^{-1}, and agar 15.0 g L^{-1}). Surfactin production was conducted utilizing mineral medium (MM), which contained (in grams per liter) $(NH_4)_2SO_4$ 1.0, Na_2HPO_4 6.0, KH_2PO_4 3.0, NaCl 2.7, and $Mg_2SO_4.7H_2O$ 0.6 [23] and 1 mL L^{-1} of a trace element solution (consisting of $ZnSO_4.7H_2O$ 10.95 g L^{-1}, $FeSO_4.7H_2O$ 5.0 g L^{-1}, $MnSO_4.H_2O$ 0.39 g L^{-1}, $CuSO_4.5H_2O$ 54 g L^{-1}, $Co(NO_3)_2.6H_2O$ 0.25 g L^{-1}, and $Na_2B_4O_7.10H_2O$ 0.17 g L^{-1}), supplemented with different carbon sources.

Clarified Cashew Apple Juice Preparation and Characterization

CAJ was obtained by compressing the cashew apple (*Anarcardium occidentale* L.). The juice was then centrifuged at 5,000×*g* for 20 min (BIO ENG, BE-6000), filtered using a 45 μm filter paper, and clarified using gelatin (10–30% *w*/*v*). The clarified CAJ (CCAJ) was characterized according to minerals composition and nitrogen, carbon, and amino acids concentration. The pH of CCAJ was determined using a potentiometer Tecnal, model Tec-3MP at approximately 27 °C. The total proteins were determined according to the Baethgen and Alley method [24], known as Kjeldahl method, and soluble proteins were assayed by the Bradford method [25]. The amino acids composition and glucose and fructose concentration were analyzed by high-performance liquid chromatography (HPLC) using a Waters high-performance liquid chromatography. Fe, Ca, Mg, Mn, Zn, and Cu were quantified by atomic absorption spectrometry using a Perkin-Elmer equipment, model To-Analyst 300. Potassium and sodium were determined for photometry flame using a DIGIMED equipment, model DM-61.

Culture Conditions

B. subtilis LAMI008 was inoculated on APGE plates and incubated at 30 °C for 24 h. After this period, three colonies were transferred to 250-mL Erlenmeyer flasks containing 50 mL of MM supplemented with different concentrations of glucose provided by clarified cashew apple juice and 0.1% of trace element. The flasks were incubated in rotary shaker (Tecnal—TE240) at 180 rpm, 30 °C for 18 h. Afterward, optical density of this culture was adjusted to 0.1 at 600 nm, and an aliquot of 10 mL (10% inoculum) was transferred to a 250-mL Erlenmeyer flask containing mineral medium and 0.1% of trace element solution, with one

of the following carbon sources: clarified cashew apple juice (MM-CCAJ) or glucose (MM-G) at a concentration of 10 g L^{-1}. The influence of yeast extract (5 g L^{-1}) supplementation to biosurfactant production was still evaluated. The experiments were carried out in rotary shaker at 180 rpm, 30°C for 120 h, and the assays were done in triplicate. Samples were collected at time-defined intervals and submitted for analysis.

Analytical Methods

Biomass Content

Cell growth was determined by measuring the optical density of samples using a UV–visible spectrophotometer (20 Genesis, BR) at 600 nm. Cell concentration was determined by dry weight of cultures filtered through previously weighted Millipore membranes, 0.45 μm pore size. Filtered samples of 5 mL culture broth under vacuum, after being washed with 5 mL of distilled water (to remove broth components), were dried until reaching constant weight [26].

Carbohydrate Concentration

Substrate concentration (glucose and fructose) was measured by HPLC using a Waters high-performance-liquid chromatographer (Waters, Milford, MA, USA) equipped with a refractive index detector (Model 2414, Waters, Milford, MA, USA) and a Shodex Sugar SC1011 column (8.0×300 mm). Water MiliQ (Simplicity 185, Millipore, Billerica, MA, USA) was used as solvent with a flow rate of 0.6 mL min^{-1} at 80 °C. The samples were identified by comparing the retention times with those of carbohydrate standards [20].

Emulsification Activity

Emulsifying activity was performed according to Cooper and Goldenberg [27] with slight modifications: 2 mL of cell free supernatant was added to 2 mL of kerosene containing 0.2 mL of pink dye and the mixture was vortexed for 2 min. After 24 h, the height of emulsion layer was measured. The emulsification index (IE_{24}) was calculated using Eq. 1.

$$IE_{24}(\%) = \frac{H_{EL}}{H_S} \times 100 \quad (1)$$

where H_{EL} is the height of the emulsion layer and H_S is the height of total solution.

Surface Tension Determination

Surface tension was determined with a Tensiometer (Torsion Balance of White Electrical Instrument) at 30 °C, according to the De Nöuy ring method. The surface tension determination was replicated and it was performed using cell free supernatants.

Surfactin Extraction

Cells were separated by centrifugation at 10,000×*g* for 15 min at 4 °C. The supernatant was filtrated using a 0.45 μm membrane (cellulose esters, Millipore), and the pH of the filtrate was adjusted to 2.0 by adding 3 M HCl. The resultant solution was maintained in repose to

allow precipitation of surfactin by 12 h. The precipitate was collected by centrifugation at 10,000×*g* for 15 min at 45 °C to obtain the crude surfactin. For further purification, the crude surfactin was dissolved in deionized water, and it was extracted three times by using an equal volume of dichloromethane (Vetec, São Paulo, Brazil) [28]. The solvent layer was harvested and evaporated to dryness. The resulting brown-colored paste was dissolved in deionized water, and it was centrifuged to remove insoluble substances. After further filtration and drying, the purified surfactin was obtained.

Determination of Surfactin Concentration

Surfactin concentration was measured by HPLC using a Waters high-performance liquid chromatographer equipped with a UV detector (Model 2487, Waters, Milford, MA, USA) at 205 nm and a Symmetry C_{18} column (150×4,6 mm, 5 μm, Waters, Ireland). The mobile phase consisted of 20% trifluoroacetic acid (3.8 mM) and 80% acetonitrile. The elution rate was 1 mL min^{-1} at 30 °C and the sample size was 20 μL. The identity of the purified surfactin was done using commercially available 95% pure surfactin (Sigma-Aldrich) as the authentic compound [28].

Fermentation Parameters

Volumetric cell productivity (P_X), expressed as $gram_x$ per liter hour, was calculated according to Eq. 2.

$$P_X = \left(\frac{\Delta X}{\Delta t}\right) = \left(\frac{X_{max} - X_0}{t}\right) \tag{2}$$

where X_{max} and X_0 are the maximum and initial values of biomass concentration, respectively, whereas t is the time needed to reach X_{max}. Volumetric biosurfactant productivity (P_p), expressed as $milligram_P$ per liter hour, was calculated according to the Eq. 3.

$$P_P = \left(\frac{\Delta P}{\Delta t}\right) = \left(\frac{P_f - P_0}{t}\right) \tag{3}$$

where P_0 and P_f are the initial and final surfactin concentrations attained after the time t. The yield of surfactin on cell mass, expressed as $milligram_P$ per $gram_x$, was calculated as the ratio of surfactin concentration (ΔP) to the corresponding biomass concentration after the same time (ΔX).

Results and Discussion

Physicochemical Characterization of Cashew Apple Juice

The physicochemical composition of cashew apple is shown in Table 1, which presents the parameters evaluated for the CCAJ. CCAJ showed high concentration of reducing sugars, mainly glucose and fructose. Moreover, the minerals, mainly iron and manganese, considered important factors in the production of surfactant by *Bacillus* [29, 30], are present in cashew apple juice.

Table 1 Composition of clarified cashew juice (CCAJ) utilized as substrate to biosurfactant production by *B. subtilis* LAMI008.

Parameters	CCAJ	Parameters (μmol/mL)	CCAJ
pH	4.32±0.0	Aspartic acid	104.04
Glucose (g/L)	43.67±0.3	Glutamic acid	146.55
Fructose (g/L)	42.43±0.1	Serine	147.90
Soluble proteins (mg/mL)	0.10±0.0	Glycine	187.60
Total proteins (mg/mL)	5.19±0.0	Histidine	26.14
Phosphorous (g/L)	1.21±0.0	Threonine	355.83
Potassium (g/L)	13.13±0.9	Alanine	242.88
Calcium (g/L)	<DL	Proline	138.41
Magnesium (g/L)	1.17±0.1	Tyrosine	73.43
Sodium (g/L)	0.09±0.0	Valine	30.66
Sulfur (g/L)	0.81±0.0	Methionine	5.08
Copper (mg/L)	<DL	Cysteine	10.38
Iron (mg/L)	6.97±2.7	Isoleucine	41.15
Zinc (mg/L)	11.20±4.3	Leucine	81.93
Manganese (mg/L)	6.40±0.4	Phenylalanine	13.05
		Lysine	11.78

The structure of surfactin is influenced by the presence of amino acids in the culture media. When amino acids are used as the sole nitrogen source, some hydrophobic amino acids insert themselves directly into selected positions of the peptide sequence, thus amplifying the original structural microheterogeneity via the production of variants [6]. Addition of glutamic and aspartic acids on media increased the lyquenesine-A production by *Bacillus licheniformis* BAS50 [31]. As it can be seen in Table 1, amino acids, considered important factors in the production of surfactant by *Bacillus*, are present in cashew apple juice (Table 1), which may also be used as nitrogen source.

In general, agroindustrial substrates containing high carbohydrate concentration are potential carbon source for biosurfactant production [32]. Accordingly, cashew apple juice appears as a suitable substrate for microorganisms' cultivation since it presents high concentration of reducing sugars, other macronutrients and micronutrients, as well as amino acids. Therefore, the exploitation of cashew apple could reduce the costs associated with biosurfactant production, representing a new application to a surplus raw material that usually spoils in soil and contributes to environmental pollution.

Evaluation of Biosurfactant Production by *B. subtilis* LAMI008 Using Mineral Media and Clarified Cashew Apple Juice (MM-CAJC) as Fermentation Media

Batch cultivation of *B. subtilis* LAMI008 was performed on mineral medium containing low levels of glucose from the CCAJ (0.25, 0.50, and 1.00 g L^{-1}). Figure 1 shows the growth profiles. It can be observed that the highest yield of biomass was obtained at 24 h of incubation for all the concentrations tested. From this time on, cell death has occurred, probably due to the shortage of the carbon source. Nevertheless, low biomass concentrations were archived, less than 0.5 g L^{-1}. To overcome this problem, glucose concentration was increased to 10 g L^{-1}.

Figure 2 shows the growth and pH profiles for *B. subtilis* LAMI008 cultivated in mineral medium supplemented with clarified cashew apple juice (MM-CCAJ) or glucose PA

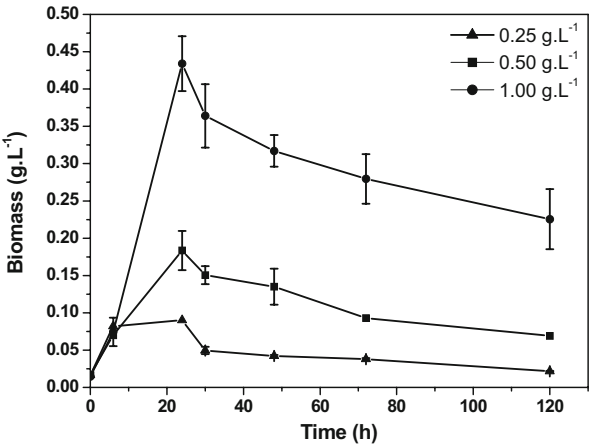

Fig. 1 Growth profile at 30 °C and 18 rpm of *B. subtilis* LAMI008 in MM formulated with different glucose concentrations from the CCAJ: 0.25, 0.50, and 1.00 g L^{-1}

(MM-G) as carbon source, both at 10 g L^{-1} of glucose. Biomass yield was 1.8-fold higher when MM-CCAJ was used probably due to fructose consumption present in CCAJ as can be deduced by diauxic growth curve. The consumption pattern of glucose and fructose during the fermentation was confirmed by monitoring these sugars (Fig. 3). After 24 h of cultivation, the acidification of both media was observed; however, at the end of cultivation, MM-G broth was more acidic than MM-CCAJ probably due to the buffer effect provided by proteins constituents (Fig. 2 and Table 1).

B. subtilis grows in the absence of oxygen using nitrate ammonification and various fermentation processes. Lactate, acetate, acetoin, ethanol, and succinate are the main fermentation products. The change to anaerobic respiration could be induced by the accumulation of acidic compounds like pyruvate and acetate from fermentation. During the process of anaerobic nitrate ammonification, nitrate is reduced by a respiratory nitrate reductase to nitrite, which is subsequently reduced further to ammonia by a general cellular nitrite reductase, which could explain the increase of the pH during the second phase of the curve when fructose was metabolized. The ability of *B. subtilis* to utilize nitrate as an alternative electron acceptor is under a complex intracellular regulation, and the postulated regulatory signal(s) is intracellular pH and/or acetate or derived metabolites [33].

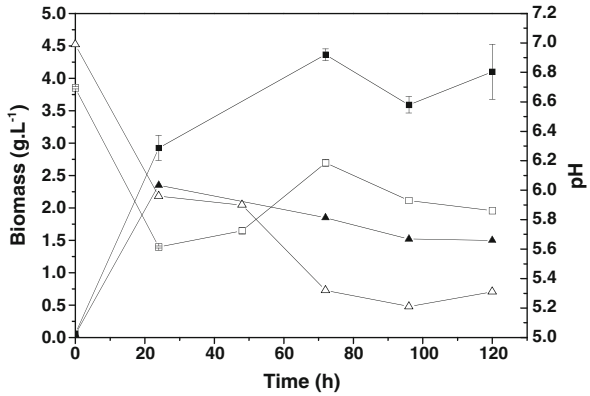

Fig. 2 Growth and pH profiles of *B. subtilis* LAMI008 batch cultivation at 30 °C and 180 rpm in MM-CCAJ and MM-G: *filled squares* biomass and *empty squares* pH in MM-CCAJ; *filled triangles* biomass and pH *empty triangles* in MM-G

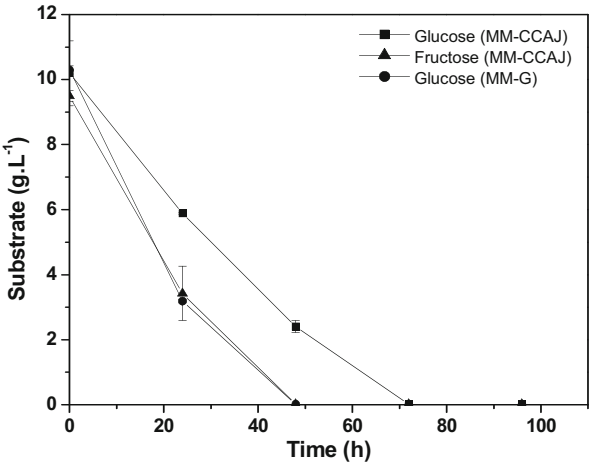

Fig. 3 Substrate consumption during *B. subtilis* LAMI008 batch cultivation at 30 °C and 180 rpm in MM-CCAJ and MM-G

B. subtilis LAMI008 produced surfactin when cultivated in MM-CCAJ with 10 g L^{-1} of glucose. Figure 4 shows the chromatograms obtained by HPLC of the surfactin purified from the fermentation broth and a commercial standard of surfactin. It can be observed that both samples shared the same retention time. The concentration of surfactin obtained was 280 mg L^{-1}, which is similar to the results (240 mg L^{-1}) obtained by other authors [13] using a minimal medium supplemented with 2% sucrose at 30 °C and pH 4.5. However, the amount of surfactin produced was not sufficient to reduce significantly the surface tension of the medium. According to the literature, the choice of nitrogen source affects biosurfactant production. Some authors [32], when studying the effects of various nutritional supplements on biosurfactant production by a strain of *B. subtilis* at 45 °C, observed that the least reduction in surface tension was achieved by organisms growing in a medium lacking a nitrogen source, which is the case of the MM-CCAJ. They also observed that surface tension reduction was better when yeast extract was used in the medium. Therefore, the effect of MM-CCAJ supplementation with yeast extract was investigated.

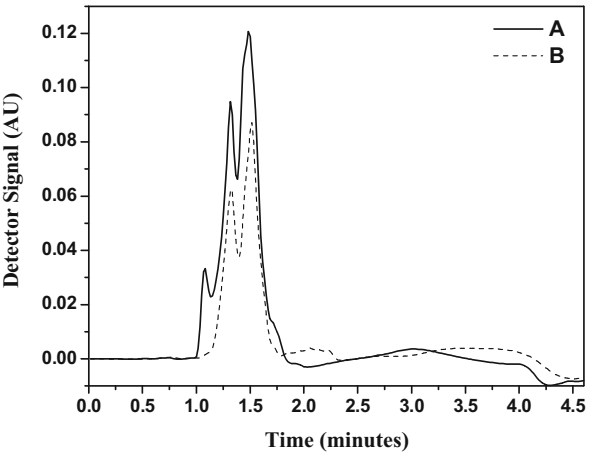

Fig. 4 Surfactin chromatogram analyzed by HPLC in Symmetry C18 column (150×4.6 mm, 5 μm, Water, Ireland) eluted with 20% acid trifluoracetic (3.8 mM) and 80% of acetonitrile at 1 mL min^{-1} and 30 °C. *A* Purified surfactin produced by *B. subtilis* LAMI008 using MM-CCAJ and *B* standard (Sigma) surfactin (0.2 mg mL^{-1})

Influence of Yeast Extract in Biosurfactant Production by *B. subtilis* LAMI008

The culture media employed for biosurfactant production by *Bacillus* strains generally present similar chemical composition, being normally supplemented with micronutrients varying, however, relative to the presence or absence of yeast extract and/or EDTA [26, 27, 32, 34]. Morán et al. [23] used yeast extract in the composition of the medium to produce surfactin and studied the effect on the biodegradation of hydrocarbons wastes, obtaining satisfactory results in the degradation of alkanes. Reis et al. [26] evaluated the influence of yeast extract, EDTA, and trace minerals in the biosurfactants production by *B. subtilis* using low-cost raw material; however, high values of surface tension were obtained in all cases, indicating that nutritional supplementation did not favor biosurfactant production although they stimulated cell growth. According to previous works, the use of yeast extract for surfactin production is contradictory [23, 26]. So, studies were conducted to verify the influence of yeast extract supplementation on surfactin production by *B. subtilis* LAMI008. Figure 5 presents growth results for *B. subtilis* LAMI008 in mineral medium containing yeast extract and the carbon sources CCAJ (MM-CCAJ) and glucose PA (MM-G) at 10 g L^{-1} of glucose concentration (Fig. 6). When glucose was used, the culture reached stationary phase after 24 h of cultivation. Biomass production increased in 50% when MM-CCAJ was used instead of MM-G, probably due to the contribution of fructose, also present in the CCAJ, on bacterial growth. In all experiments, there were decreases in pH of the medium, values ranging from 5.2 to 6.0. Fox and Bala [35] observed an increase in pH values of different non-buffered potato media ranging from 6.2 to 8.5 to produce surfactin using *B. subtilis*. In this work, the results were different, probably due to the buffer effect provided by proteins present in the clarified cashew apple juice (Fig. 5).

The addition of yeast extract increased cell growth by 25% (Figs. 2 and 5) because it contains complex B vitamins, which are necessary to form enzymes and coenzymes, and also amino acids and other cell growth-stimulating compounds [26], which may contribute to biosurfactant production.

The volumetric cell productivities obtained with MM-G and MM-CCAJ, without supplementation, were 0.092 and 0.117 g_x/L h, respectively, after 24 h of fermentation. However, the volumetric cell productivities using media supplemented with yeast extract

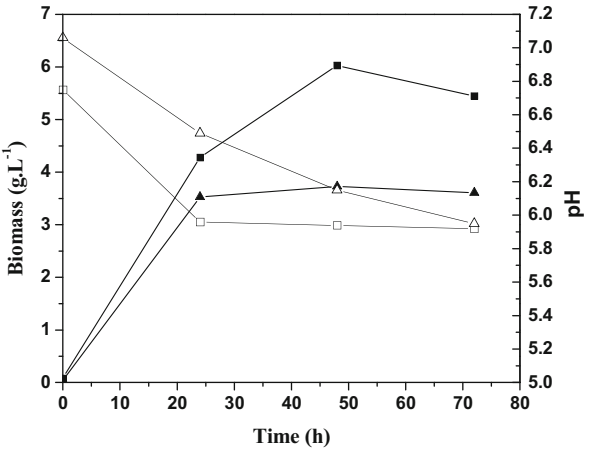

Fig. 5 Growth and pH profile during biosurfactant production by *B. subtilis* LAMI008 in media supplemented with yeast extract (5 g L^{-1}): *filled squares* biomass and *empty squares* pH in MM-CCAJ; *filled triangles* biomass and *empty triangles* pH in MM-G

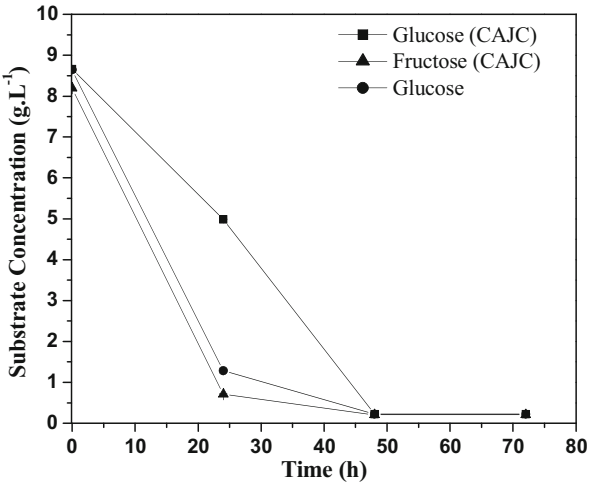

Fig. 6 Substrate consumption during biosurfactant production by *B. subtilis* LAMI008 in media supplemented with yeast extract (5 g L^{-1}): glucose from CCAJ (*squares*), fructose from CCAJ (*triangles*), glucose PA (*circles*)

were 0.142 and 0.175 g_x/L h for MM-GY and MM-CCAJY, respectively, an increase of around fivefold in productivity, for both cases, when yeast extract was used.

Table 2 presents biosurfactant production in mineral medium, supplemented or not with yeast extract, using CCAJ or glucose PA, as carbon sources. Both carbon sources tested have favored extracellular production of active surface agent by *B. subtilis* LAMI008, which was estimated by the reduction in surface tension of the fermented broth free of cells. Based on the obtained results, CCAJ is a suitable substrate for microbial growth and biosurfactant biosynthesis.

The lowest values of surface tension using MM-CCAJ and MM-G were obtained after 24 h of cultivation in the presence of yeast extract. This time coincides with the consumption of substrate and the beginning of a stationary growth phase. The synthesis of the surface-active agent took place in the late-exponential phase, achieving its maximum value at beginning of the stationary growth phase. Sheppard and Mulligan [36] described that surfactin production by *B. subtilis* ATCC 21332 occurred mainly in the mid- to late-exponential phase of growth, similar result obtained in this work. However, Kim et al. [4] observed a direct relationship among cellular growth, biosurfactant production, and substrate consumption when *B. subtilis* C9 was cultivated in synthetic medium.

It can be observed in Table 2 that no reduction in surface tension was obtained without yeast extract supplementation, using cashew apple juice or glucose as carbon sources. This

Table 2 Effect of different carbon sources on growth and biosurfactant production by *B. subtilis* LAMI008 in mineral media supplemented or not with yeast extract (YE).

Carbon source	Media	YE (g L^{-1})	Surface tension initial (dyn cm^{-1})	Surface tension (dyn cm^{-1})[a]	Reduction of surface tension (%)
CCAJ	MM-CCAJ	0.0	60.45±0.00	60.45±0.00	0.00
Glucose	MM-G	0.0	56.50±0.58	56.72±0.53	0.00
CCAJ	MM-CCAJ	5.0	50.30±0.24	39.55±0.45	21.37
Glucose	MM-G	5.0	58.00±0.00	44.00±0.00	24.14

[a] After 24 h of fermentation

result is contradictory with the ones obtained by Fox and Bala [35] and Reis et al. [26], but is in agreement with the one obtained by Makkar and Cameotra [32].

The lowest value of surface tension (39.55 dyn cm^{-1}), after 24 h of cultivation, was obtained when using MM-CCAJ, with a reduction of 21.37% of its initial value. A surfactin concentration of 3,500±8.0 mg L^{-1} of culture medium was obtained when MM-CCAJ was supplemented with yeast extract (MM-CCAJY). Yeh et al. studied the addition of a small quantify of solid porous carriers, e.g., activated carbon (25 g L^{-1}) and expanded clay (133 g L^{-1}), and obtained a surfactin yield of 3,600 and 3,300 mg L^{-1}. Wei and Chu related that surfactin productivity by *B. subtilis* was increased from 330 to 2,600 mg L^{-1} by the addition 0.01 mM Mn^{2+} to a defined glucose medium. In this work, surfactin concentration in the broth was enhanced from 280 to 3,500 mg L^{-1} when yeast extract was added to MM-CCAJ. This result demonstrates a feasible way to produce surfactin from *B. subtilis* using clarified cashew apple juice, an agroindustrial residue, as carbon source.

Volumetric surfactin productivity and surfactin yield on the absence of yeast extract (MM-CCAJ) were 5.83 mg_P/L h and 62.22 mg_P/g_x, respectively. On the other hand, when MM-CCAJ was supplemented with yeast extract (MM-CCAJY), surfactin productivity and yield were enhanced to 72.92 mg_P/L h and 564.52 mg_P/g_x, respectively. Other authors [9] obtained a volumetric biosurfactant productivity of 121 mg_P/L h and biosurfactant yield of 58 mg_P/g_x on batch cultivations of *Bacillus atrophaeus* ATCC 9372, using glucose (2.5 g L^{-1}) and casein (10 g L^{-1}). When glucose concentration was enhanced to 18 g L^{-1}glucose, biosurfactant productivity and yield were 191 mg_P/L h and 256 mg_P/g_x, respectively.

The emulsification activity was monitored during the course of surfactin fermentation by determining the index of emulsification (IE_{24}) for kerosene with the supernatant of the culture free of cells. Figure 7 shows the emulsification activity of the biosurfactant produced by fermentation in MM-CCAJY at 30 °C. After 72 h of cultivation, 65% of kerosene emulsification was obtained, indicating that this biosurfactant has an emulsifying activity.

Identification of the emulsification activity of surfactin produced from *B. subtilis* LAMI008 is thus in great demand to assess the applicability of the biosurfactant in enhanced oil recovery or oil pollution bioremediation. It is suggested that the surfactin produced in this work can be applied in bioremediation of oil or hydrophobic pollutants.

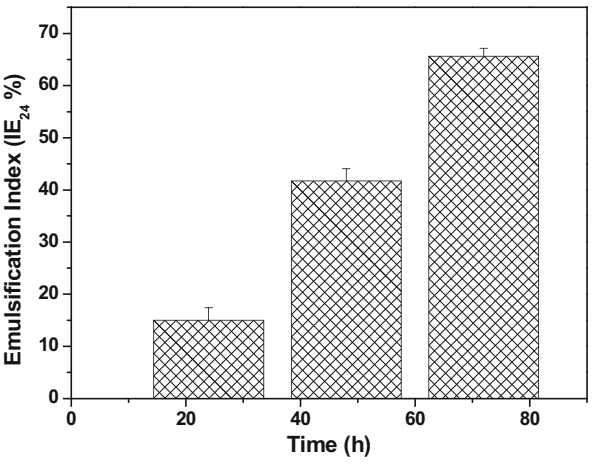

Fig. 7 Emulsifying activity (IE_{24}%) of the biosurfactant produced by *B. subtilis* at 30 °C using cashew apple juice supplemented with yeast extract (MM-CCAJY) as culture medium. Error bars represent standard deviations

Conclusion

The results of this work showed that the clarified cashew apple juice represents an alternative and viable substrate for surfactin production, a surfactant of great commercial value. *B. subtilis* LAMI008 was capable of growing and producing biosurfactant in the different cultivation mineral media supplemented with yeast extract and with clarified cashew apple juice or glucose PA, as carbon sources. Analyses of the culture supernatants along cultivation time showed that there was surfactin production, reaching a maximum concentration of 3,500 mg L^{-1}. Furthermore, the emulsification index (IE_{24}) value of the biosurfactant was found to be 65%, demonstrating its capacity to emulsify kerosene. Based on the results, MM-CCAJY is a suitable substrate for growth and surfactin production by *B. subtilis* LAMI008.

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