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Cassava Flour Wastewater as a Substrate for Biosurfactant Production

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Abstract

Five cassava flour wastewater (*manipueira*) preparations were tested as culture media for biosurfactant production by a wild-type *Bacillus* sp. isolate. No-solids (F), no-solids diluted (F/2), natural (I), natural diluted (I/2), and decanted (IPS) were the tested *manipueira* media. The microorganism was able to grow and to produce biosurfactant on all *manipueira* preparations. The media whose solids were removed (F and F/2) showed better results than preparations with the presence of solids (I, I/2, and IPS). No-solids medium (F) showed a surface tension of 26,59 mN/m and reciprocal of critical micelle concentration of over 100 and was selected as a potential substrate for biosurfactant production.

Index Entries: Biosurfactant; *Bacillus* sp.; cassava flour wastewater.

Introduction

Surface-active molecules produced by microorganisms, also called biosurfactants, are of great interest as substitutes for chemically derived surfactants mainly because of their low toxicity and biodegradability *(1,2)*. Biosurfactants find applications in the cosmetic, pharmaceutical, and food industries as emulsifiers, humectants, dispersants, and detergents *(3,4)*. Moreover, they are ecologically safe and suited for environmental applications such as bioremediation, dispersion of oil spills, and waste treatment *(5,6)*. Although interest in biosurfactants is increasing, these compounds are not competitive with synthetic surfactants owing to high production costs *(7)*. The use of alternative substrates (substitutes for conventional media), usually renewable resources, could be explored since substrates account for 50% of final product costs *(8)*.

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Selecting an alternative resource involves finding a residue with the right balance of nutrients to support optimal growth and production *(8,9)*. Agroindustrial residues with high contents of carbohydrates or lipids meet the requirements for use as substrates for biosurfactant production. Peat hydrolysate, olive oil mill effluent, lactic whey, and potato process effluents are some examples of low-cost substrates that have been reported for biosurfactant production *(10–14)*.

One of the biggest problems faced by cassava flour factories in Brazil is the disposal of *manipueira*, a liquid waste generated in large amounts (300 L/t of processed roots) *(15)*. The disposal of *manipueira* causes environmental problems owing to its high organic load, although this waste is composed of carbohydrates, nitrogen, minerals, and trace elements and therefore has potential as a substrate for the biotechnological processes. In the present study, we investigated the production of biosurfactant by a *Bacillus* sp. isolate using different *manipueira* preparations as substrates.

Materials and Methods

Microorganisms

A wild-type *Bacillus* sp. LB5a isolate selected for its capacity to decrease surface tension on *manipueira* medium *(16)* was maintained on nutrient agar (Difco, Detroit, MI) slants at 4°C.

Preparation of Substrate

Manipueira from the manufacture of cassava flour was collected at São Paulo State and stored at –18°C until needed. The composition of *manipueira* waste utilized is given in Table 1. Five different media were prepared from the original waste and autoclaved at 121°C for 15 min:

1. Natural *manipueira* medium (I) with the presence of insoluble solids (6.2% [w/v] total solids) was dispersed and distributed in Erlenmeyer flasks. The initial pH was 5.9.
2. Naturally diluted *manipueira* (I/2) was natural *manipueira* diluted 1:2 with distilled water (3.8% [w/v] total solids). The initial pH was 5.8.
3. Decanted *manipueira* (IPS) was natural *manipueira* waste maintained for 1 h at room temperature to permit the decantation of solids. The upper liquid was gently removed and distributed in flasks (2.3% [w/v] total solids). The initial pH was 5.8.
4. No solids *manipueira* (F) was prepared by heating the waste to boiling point to remove all the solids. After cooling, the substrate was centrifuged at 8000*g* for 20 min in a centrifuge (model J2-21; Beckman). The supernatant was distributed in flasks. The initial pH was 5.8.
5. No solids diluted *manipueira* (F/2) was no solids *manipueira* diluted 1:2 with distilled water. The initial pH was 5.9.

Table 1
Composition of Cassava
Flour Wastewater (*Manipueira*)

Parameter	Value
Total solids (g/L)	62.0
Total carbohydrates (g/L)	41.5
Reducing sugars (g/L)	18.2
No reducing sugars (g/L)	23.2
Total nitrogen (g/L)	2.1
Phosphorus (mg/L)	244.5
Potassium (mg/L)	3472.6
Calcium (mg/L)	292.5
Magnesium (mg/L)	519.0
Sulfur (mg/L)	154.0
Iron (mg/L)	7.8
Zinc (mg/L)	2.8
Manganese (mg/L)	1.7
Copper (mg/L)	1.0
pH	5.8

Inoculum and Culture Conditions

The bacterial isolate was streaked in a nutrient agar slant and incubated for 24 h at 30°C. A loop of culture was inoculated in 20 mL of nutrient broth (Difco) in a 50-mL Erlenmeyer flask and incubated in a rotary shaker (model G25R; New Brunswick) for 7 h, at 150 rpm and 30°C. An aliquot of 1 mL of inoculum was transferred to 15 mL of *manipueira* medium in a 50-mL Erlenmeyer flask and incubated at 30°C and 150 rpm in a rotary shaker (model G25R; New Brunswick). Samples were collected at set time intervals and submitted for analysis. The experiments were conducted in three independent replicates.

Analytical Measurements

Samples were submitted to serial dilutions and viable counts were performed by spread plate technique. Total carbohydrates were estimated using the phenol-sulfuric assay *(17)*.

Culture samples were centrifuged at 8000*g* for 20 min for cell removal. The supernatant was submitted to surface tension critical micelle dilution (CMD) and reciprocal of critical micelle concentration (CMC^{-1}) analysis. Surface tension was determined with a Krüss Processor Tensiometer (model K12 T) using the plate method. For CMD measurements, the cell-free broth was diluted 10 (CMD^{-1}) and 100 times (CMD^{-2}) with distilled water, and the surface tension of these dilutions was determined. The maximum SD allowed for surface tension measurements was 0.20.

Table 2
Comparison of Data Obtained from Different *Manipueira* Media

Medium	Time (h)	Total carbohydrates (g/L)	Viable count (ufc/mL)	Surface tension (mN/m)[a]	CMD^{-1} (mN/m)[b]	CMD^{-2} (mN/m)[b]	Crude biosurfactant (g/L)
I	0	43.57	1.0×10^7	44.04	54.08	66.49	—
	24	24.65	1.8×10^9	31.57	34.56	48.07	1.23
	48	15.95	1.36×10^9	29.86	31.99	41.04	1.84
	72	9.58	7.0×10^8	29.14	29.66	37.97	1.69
I/2	0	27.93	1.0×10^7	46.57	57.06	67.43	—
	24	11.74	1.68×10^9	29.16	31.05	44.09	0.90
	48	6.44	9.8×10^8	29.37	31.22	41.31	1.00
	72	5.50	1.47×10^9	28.13	29.07	36.86	1.36
F	0	30.24	1.0×10^7	49.47	54.94	59.45	—
	24	8.22	1.48×10^9	26.57	27.24	34.05	2.34
	48	4.99	9.3×10^8	26.87	27.20	32.42	3.17
	72	4.60	1.32×10^9	26.59	26.92	32.06	2.74
F/2	0	18.24	1.0×10^7	51.31	57.42	64.16	—
	24	4.25	4.8×10^8	26.47	27.46	36.38	1.05
	48	4.04	7.1×10^8	26.67	27.43	35.89	1.29
	72	3.44	1.15×10^9	26.63	27.45	35.85	1.32
IPS	0	44.36	1.0×10^7	45.24	51.41	59.54	—
	24	15.63	2.25×10^9	29.89	31.48	40.45	1.33
	48	8.92	6.6×10^8	27.9	28.8	36.7	1.81
	72	8.38	1.48×10^9	27.51	27.99	34.74	2.23

[a]Maximal SD of 0.20.
[b]CMD^{-1} = CMD 10X; CMD^{-2} = CMD 100X.

The critical micelle concentration (CMC) is a useful index for evaluating surface activity and is defined as the surfactant concentration at which an abrupt increase in surface tension is observed *(18)*. The CMC^{-1} is the dilution factor required to reach the CMC and was determined by measuring the surface tension of serial diluted broth samples as described by Sheppard and Mulligan *(10)*.

Biosurfactant was isolated from cell-free broth by precipitation after adjusting broth pH to 2.0 using 6 *N* HCl and keeping it at 4°C overnight. The precipitate thus obtained was pelleted at 8000*g* for 20 min, dried, and weighed.

Results

The data obtained during cultivation of *Bacillus* sp. LB5a in tested cassava wastewater media are presented in Table 2. The microorganism was able to grow and produce biosurfactant in all tested media. Growth patterns were similar; after 24 h cells reached stationary phase. Total car-

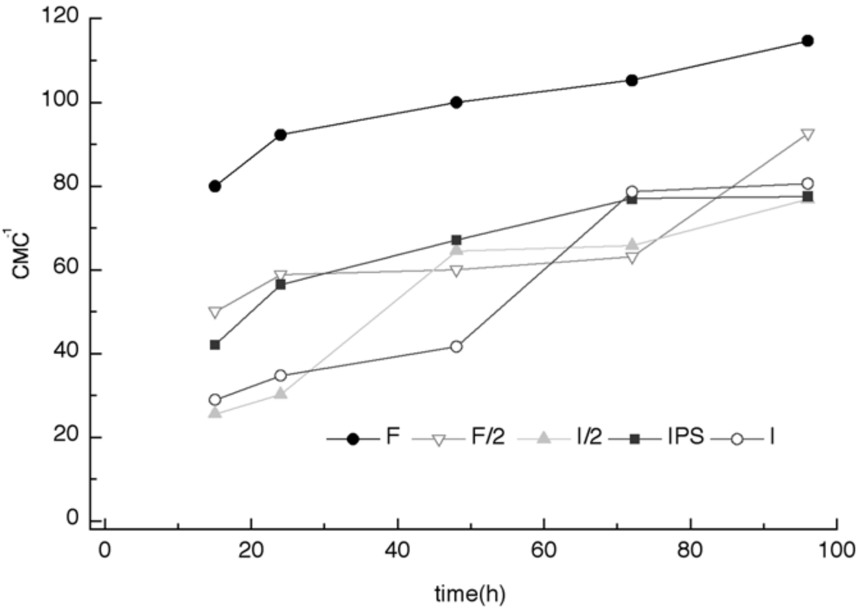

Fig. 1. Time course of CMC^{-1} on *manipueira* media.

bohydrates consumption was about 80% for all media tested, with most of the carbohydrates (>50%) being consumed during the first 24 h of fermentation. No-solids media (F and F/2), which had a lower carbohydrate content, demonstrated the lowest surface tension results, whereas the high carbohydrate content of media I and IPS did not significantly increase cell growth or surface tension. No-solids media showed a surface tension of 26.57 mN/m after 24 h, while on solid-content media (I, IPS, and I/2) the lowest surface tension attained was 27.5 mN/m after 72 h (IPS).

CMD^{-1} and CMD^{-2} data were helpful as an indirect indication of surfactant concentration on culture media *(19)*. The results obtained from medium F after 72 h showed a CMD^{-1} of 26.92 mN/m and a CMD^{-2} of 32.06 mN/m, whereas for F/2, although the surface tension was similar, the values of 27.43 mN/m (CMD^{-1}) and 35.82 mN/m (CMD^{-2}) suggested that surface activity was higher on F medium. In fact, the crude biosurfactant concentration obtained on *manipueira* F corroborates this statement.

More than 55% of crude biosurfactant was produced during the first 24 h of cultivation, and surface tension reached minimum levels in approx 48 h. After 48 h, biosurfactant production was considerably reduced and even a decrease in biosurfactant concentration was observed for media I and F. The decrease in surface activity also occurred at minor rates and could be observed by CMD data.

The CMC^{-1} obtained for the tested media is shown in Fig. 1. *Manipueira* media with solids contents reached approximately the same values of CMC^{-1} after 96 h while no-solids media showed the highest values. Time course evaluation indicated that F medium had the highest surface activity and at approx 15 h, the CMC^{-1} was about 80, reaching 105 after 72 h. After 96 h, a CMC^{-1} of 114 was attained; therefore, culture broth should be diluted 114 times to reach 0.87% (v/v) CMC.

Discussion

The carbohydrates present in *manipueira* were consumed, and the levels of iron and manganese, important factors for biosurfactant production by *Bacillus (20,21)*, suggested that the *manipueira* medium is a suitable substrate for supporting cell growth and biosurfactant accumulation by *Bacillus* sp. LB5a.

Biosurfactant production by *Bacillus* sp. LB5a on *manipueira* media seemed to be growth associated because the bulk of product accumulation (>55%) occurs during the first 24 h when cell growth reached the maximum level.

The CMC^{-1} is a direct indication of surface activity in the broth; the higher the CMC^{-1} the greater the surface activity. This is, therefore, a more important measurement than the actual quantity of surfactant if the surfactant characteristics vary from cultural conditions. From CMC^{-1} data it is clear that medium F showed the highest surface activity of all the tested media, and although medium F/2 also showed good results, the dilution of *manipueira* F waste was not necessary.

The results suggest that the presence of solids in *manipueira* waste is inversely related to broth surface tension. Thompson et al *(13)*, using a *Bacilllus subtilis* strain, also observed that a high-solids potato process effluent showed lower biosurfactant production than a low-solids effluent. Few differences in media appear to influence the characteristics of the surface-active compounds produced, as reflected by the different CMC^{-1} curves. The composition of culture medium was previously described as one of critical importance for determining product yield and biosurfactant properties *(10)*.

The medium F prepared from *manipueira* offers promise as a substrate for biosurfactant production by the isolate *Bacillus* sp. LB5a. The tested solids contents media demonstrated high surface tension and low CMC^{-1} values and are not recommended for biosurfactant production.

Conclusion

This initial study indicates that *manipueira* could be used as an alternative substrate for biosurfactant production and the no-solids medium preparation (F) presented the highest potential as a culture medium. The utilization of cassava flour wastewater could reduce environmental problems for processing industries while decreasing the estimated cost of biosurfactant production.

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