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Sugarcane Bagasse as Raw Material and Immobilization Support for Xylitol Production

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Abstract

Xylose-to-xylitol bioconversion was performed utilizing *Candida guilliermondii* immobilized in sugarcane bagasse and cultured in Erlenmeyer flasks using sugarcane bagasse hydrolysate as the source of xylose. Fermentations were carried out according to a factorial design, and the independent variables considered were treatment, average diameter, and amount of bagasse used as support for cell immobilization. By increasing the amount of support, the xylitol yield decreased, whereas the biomass yield increased. The diameter of the support did not influence xylitol production, and treatment of the bagasse with hexamethylene diamine prior to fermentation resulted in the highest amount of immobilized cells.

Index Entries: Xylitol; cell immobilization; sugarcane bagasse; *Candida guilliermondii*; hemicellulosic hydrolysate.

Introduction

Sugarcane bagasse is an abundant agroindustrial byproduct. It is a fibrous lignocellulosic material generated by the sugar industry from cane stalks after juice extraction. Annually, the worldwide production of this byproduct is about 234 million t *(1)*, and a major part of it is used as an energy source in the sugarcane industry. However, there is an excess of bagasse and some alternatives for its use have been researched, including the production of alcohol and alkaloids, mushrooms, protein-enriched animal feed, and enzymes *(2)*. Xylitol bioproduction from the xylose contained in the hemicellulosic fraction of this residue is another alternative to add value to the raw bagasse.

Xylitol is a polyalcohol industrially used owing to its sweetening power, non- and anticariogenicity, and microbial growth inhibition capacity

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(3–5). Currently, it is produced under conditions of high pressures and temperatures by chemical reduction of pure xylose obtained from hemicellulosic hydrolysates of wood. Because of the drastic conditions used and the need for xylose purification prior to conversion, this chemical process is expensive and limits the use of xylitol on a larger scale.

To reduce production costs, other processes have been studied for xylitol production, and the fermentation of hemicellulosic hydrolysates from agricultural residues and byproducts is a promising method *(6–8)*. In the biotechnological process, mild conditions of temperature and pressure are employed, and the hydrolysate can be directly used for bioconversion without previous purification of xylose *(9,10)*. Many alternatives have been researched for this bioprocess, and, during the last 10 yr, immobilized cell systems have also been evaluated *(11–13)*.

The use of immobilized cells is advantageous in bioprocesses because it facilitates cell reutilization, enables better control of rheologic medium properties and allows the use of high biocatalyst densities and the operation in continuous mode using high dilution rates without bioreactor washout *(14–16)*. However, to obtain good performance using systems with immobilized cells, the right choice of cell carrier is essential. A good carrier would be a material that possesses the following qualities:

1. Keeps its physical integrity under the fermentation conditions.
2. Does not reduce the activity of the cells.
3. Has high superficial area and high diffusion coefficient for substrates and products.
4. Is resistant to microbial and chemical degradation.
5. Is available in suitable quantities.
6. Is inexpensive *(17)*.

By these criteria, sugarcane bagasse seems to be a suitable material for cell immobilization, mainly owing its great availability at low cost.

In this context, the present work dealt with the utilization of sugarcane bagasse as a carrier for immobilization of *Candida guilliermondii* and as a raw material for the production of xylose-rich hemicellulosic hydrolysate. The immobilized yeasts were cultured in Erlenmeyer flasks, aiming to convert the xylose present into the hydrolysate in xylitol.

Materials and Methods

Preparation and Treatment of Sugarcane Bagasse Hydrolysate

Sugarcane bagasse was obtained from Usina Guarany (Olímpia, SP, Brazil). This material was used for the hemicellulosic hydrolysate preparation in a 250-L stainless steel reactor, loaded with bagasse and a sulfuric acid solution (100 mg of acid/g of dry matter). The reactor was operated at a solid/liquid ratio of 1:10 at 121°C for 10 min under agitation

at 50 rpm. Afterward, the hemicellulosic hydrolysate was concentrated in a 6-L vacuum evaporator at 70 ± 5°C to obtain a xylose concentration of about 40 g/L and then detoxified as described by Alves et al. *(18)*.

Microorganism and Inoculum Cultivation

The experiments were carried out using cells of *C. guilliermondii* FTI 20037, as described by Barbosa et al. *(19)*, which were kept at 5°C on malt extract agar slants. The inoculum was grown by transferring a loopful of a slant culture of the yeast to 125-mL Erlenmeyer flasks containing 50 mL of growth medium composed of 30 g/L of xylose, 3.0 g/L of $(NH_4)_2SO_4$, 0.10 g/L of $CaCl_2 \cdot 2H_2O$, and 10% (v/v) rice bran extract. The flasks were maintained under agitation at 200 rpm and 30°C for 24 h. Then their content was centrifuged (2200*g*, 20 min), and the cell pellet was rinsed twice with sterile water before immobilization.

The rice bran extract was prepared by autoclaving at 110°C for 15 min a suspension of rice bran in water (200 g/L). This suspension was then centrifuged (2200*g*, 20 min), and the liquid was used in the experiments.

Preparation of Carrier

Sugarcane bagasse was crushed and classified according to its diameter by using standard Tyler sieves with mesh numbers of 14, 16, 20, 24, and 35. The crushed and classified bagasse was washed with distilled water and dried at 105°C. This material was used, untreated or treated, as support for cell immobilization.

The bagasse treatment procedure was adapted from Tyagi et al. *(20)* and Porath and Fornstedt *(21)*: Ten grams of bagasse (dry mass) was suspended in 240 mL of 1 *M* NaOH, and, then 30 mL of epichlorohydrin was added. The mixture was heated at 60°C for 2 h at 200 rpm. Excess epichlorohydrin was removed by extensive washing with distilled water until no odor could be detected, and the bagasse was dried at 105°C for 24 h. Following this, the dried material was suspended in 100 mL 0.01 *M* NaOH, 30 mL of 85% hexamethylene diamine was added, and the suspension was heated at 60°C for 2 h at an agitation of 200 rpm. This mixture was allowed to cool at –4°C for 12 h. Then the liquid was removed, the solid material was washed with distilled water, and the treated bagasse was dried at 105°C for 24 h.

Cell Immobilization and Fermentation Conditions

Cells were immobilized *in situ* in the fermentation flasks by natural adsorption onto the untreated or treated bagasse at the beginning of fermentation. The fermentation medium was composed of the hydrolysate supplemented with 0.1 g/L of $CaCl_2$, 3 g/L of $(NH_4)_2SO_4$, and 10% (v/v) rice bran extract. The fermentation runs were carried out in 125-mL Erlenmeyer flasks containing the carrier and fermentation medium in a

Table 1
Coded Levels of Selected Variables[a]

Variable	Level	
	–	+
X_1 (g)	0.5	1.5
X_2 (mm)	0.559[b]	1.080[b]
[mesh no., standard Tyler sieves]	[–24/+35]	[–14/+16]
X_3	Untreated	Treated

[a]X_1, carrier mass; X_2, carrier average diameter; X_3, treatment.
[b]Arithmetic average between the opening of the two sieves.

working volume of 50 mL. The flasks were inoculated with 1 g/L of yeast and maintained at 30°C for 48 h at an agitation of 200 rpm.

Experimental Design

Table 1 gives the levels of the independent variables selected for study. A complete 2^3 factorial design with experiments at the central points was utilized, and the results were analyzed by Statistica 5.1 software (StatSoft, Tulsa, OK). The response variables considered were xylitol yield ($Y_{p/s}$), cell yield ($Y_{x/s}$), volumetric xylitol productivity (Q_p), and yield of immobilization (η_{imob}).

Analytical Methods

Xylose and xylitol concentrations were determined by high-performance liquid chromatography using a Bio-Rad HPX87H (300 × 7.8 mm) column with a refractive index detector. Determinations were performed at 45°C using 0.01 *N* H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min and a sample injection volume of 20 µL.

Free cell concentration was determined by optical density measurements at 600 nm. The mass of immobilized cells at the end of the fermentations was estimated by the difference between the dry weights of the carrier at the beginning and at the end of the fermentations. The concentration of immobilized cells in the reactor was determined by dividing the mass of immobilized cells by the total reactor working volume, including the volume of fermentation medium and carrier.

Xylitol yield ($Y_{p/s}$) (g/g) was considered as the ratio between xylitol production (g/L) and xylose consumption (g/L), and xylitol productivity (Q_P) (g/[L · h]) was the ratio between xylitol production (g/L) and fermentation time (h). Yield of immobilization (η_{imob}) was considered as the ratio between the immobilized and the total cell concentration at the end of each batch.

Results and Discussion

A treatment similar to that applied on sugarcane bagasse in the present work was initially used by Porath and Fornstedt *(21)* for preparation of arginine-agarase and sulfanilic acid–agarose for the fractionation of human serum by employing a technique called pulse elution. Later, Tyagi et al. *(20)* employed this treatment using hexamethylene diamine on sugarcane bagasse, aiming to immobilize *Saccharomyces cerevisiae* for continuous ethanol production. In the present work, the treatment by Tyagi et al. *(20)* was employed to enhance the cell loading of *C. guilliermondii* on the sugarcane bagasse for the purpose of producing xylitol. As can be observed in Fig. 1, besides the chemical modification, the treatment produced structural changes on the material's surface. The organized and typical fibrous structure of the bagasse was modified as a result of this chemical treatment.

Immobilization of the yeast cells occurred by natural adsorption *in situ*, at the beginning of the fermentation, and did not require a separate immobilization procedure, allowing product formation and cell immobilization at the same time. Santos et al. *(22)*, working with cells of *C. guilliermondii* FTI 20037, studied immobilization *in situ* on porous glass beads in a fluidized-bed reactor and obtained a high cell concentration in the reactor after the fermentation. In the present work, this procedure resulted in a higher immobilized cell concentration. As can be observed in Table 2, the highest immobilization yield (43%) was obtained by using 1.5 g of the treated bagasse with an average diameter of 0.559 mm. On the other hand, the highest xylitol production (20.14 g/L) was obtained in the experiment under a low level of carrier mass and with higher diameter. Under this condition, the xylitol yield (0.59 g/g) was the highest, and the cell yield (0.11 g/g) was the lowest, suggesting that the yeast metabolism was directed to xylitol production instead of the formation of biomass. Moreover, under this condition, the immobilization yield was low (13%). Thus, the condition that favors high cell loading is not the one that promotes the highest xylitol yield.

The yield of immobilization was significantly (95% confidence level) influenced by the mass of carrier and its treatment with epichlorohydrin and hexamethylene diamine. An increase in the mass of the bagasse used for immobilization from 0.5 to 1.5 g resulted in an average increase of about 18% on the yield of immobilization (Table 3). Regarding treatment of the support, when it was applied, the average increase in the yield of immobilization was 9%. In fact, the treatment resulted in a cell loading higher than that observed when untreated bagasse was used. Figure 2 shows that the cell adhesion was more efficient when the treated bagasse was used.

Xylitol and biomass production were influenced by the mass of carrier and its treatment (Table 3). An increase in the mass of bagasse resulted in a significant (95% confidence level) decrease in xylitol yield. This effect can

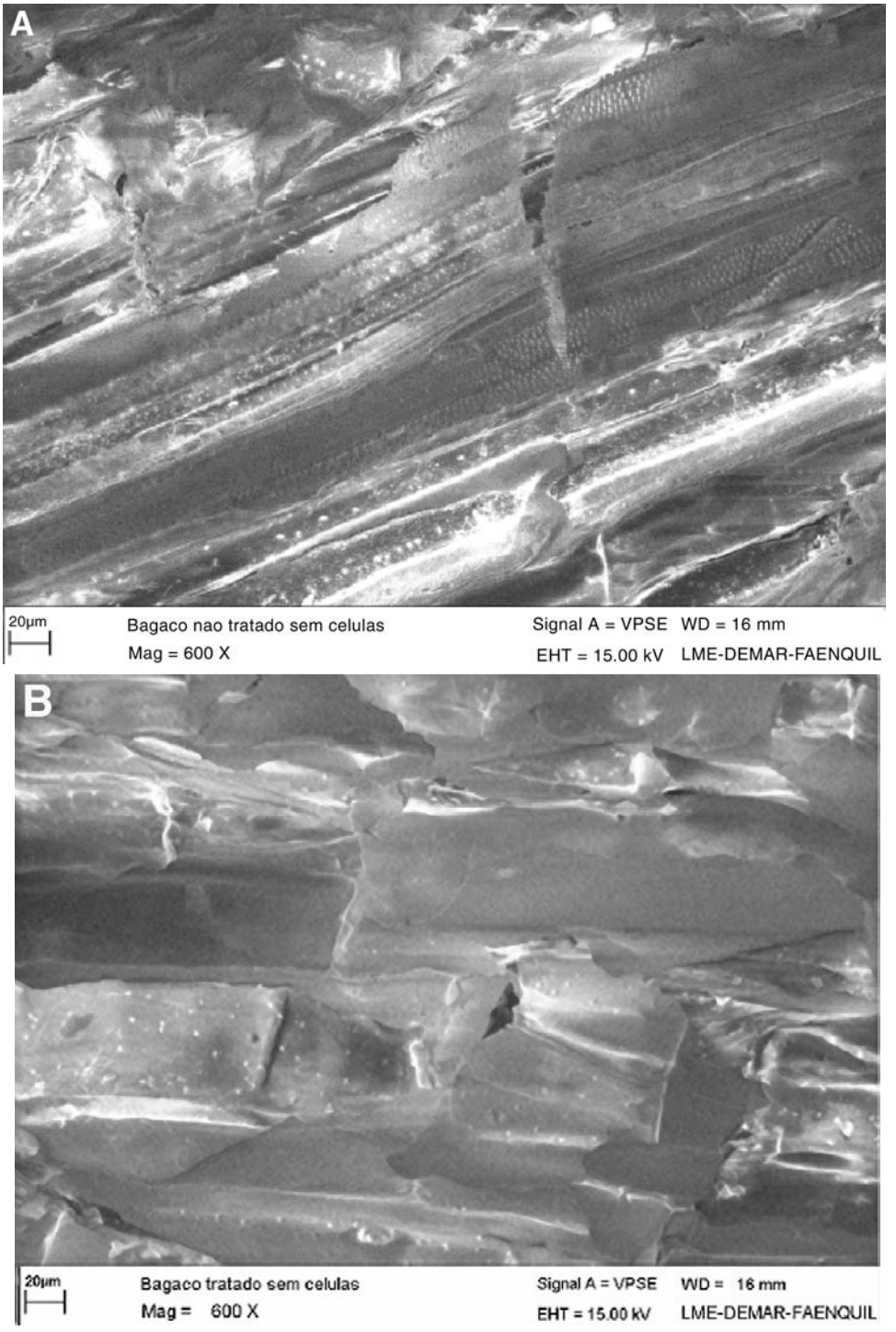

Fig. 1. Micrograph of **(A)** raw sugarcane bagasse and **(B)** treated sugarcane bagasse, obtained before cell adhesion by scanning electron microscopy (SEM) (average diameter of bagasse: 1.080 mm; magnification: 600-fold).

Table 2
Evaluation of Xylitol Production from Sugarcane Bagasse Hemicellulose Hydrolysate by *C. guilliermondii* Cells Immobilized on Sugarcane Bagasse[a]

Experiment (coded value)			P	Q_s	Q_p	$Y_{p/s}$	X	$Y_{x/s}$	η_{imob}
X_1	X_2	X_3	(g/L)	(g/[L·h])	(g/[L·h])	(g/g)	(g/L)	(g/g)	(%)
–1	–1	–1	16.05	0.7	0.33	0.47	5.424	0.15	7.63
1	–1	–1	12.77	0.74	0.27	0.48	6.648	0.25	23.89
–1	1	–1	17.01	0.59	0.35	0.6	5.406	0.16	8.44
1	1	–1	11.47	0.62	0.24	0.39	7.404	0.27	17.88
–1	–1	1	19.24	0.72	0.4	0.56	5.226	0.11	15.04
1	–1	1	14.28	0.88	0.4	0.45	6.834	0.29	43.37
–1	1	1	20.14	0.71	0.42	0.59	5.09	0.11	12.97
1	1	1	18.69	0.77	0.39	0.51	8.6	0.2	30.12
0	–0.2	–1	15.11	0.67	0.31	0.47	6.236	0.2	14.85
0	–0.2	–1	14.35	0.69	0.3	0.43	5.712	0.16	11.41
0	–0.2	1	19.04	0.75	0.4	0.53	6.928	0.16	22.34
0	–0.2	1	19.76	0.75	0.41	0.55	6.066	0.13	16.25

[a]P, final xylitol concentration; Q_s, volumetric xylose consumption rate; Q_p, xylitol volumetric productivity; $Y_{p/s}$, xylitol yield; X, final total cell concentration; $Y_{x/s}$, cell yield; η_{imob}, yield of immobilization.

be explained by the presence of more solids in the medium. The high concentration of solids in suspension in the liquid could favor higher oxygen dissolution into the medium, promoting a deviation of metabolism from xylitol to the formation of biomass. In fact, the average reduction in xylitol yield (effect of about 0.1 g/g, as shown in Table 3) corresponded to a similar average increase in cell yield (Table 3). The deviation of metabolism from xylitol production to the formation of biomass under conditions of higher oxygen availability is reported in other works *(11,23)*.

Xylitol productivity was significantly influenced by the treatment of the carrier. When the treated bagasse was used as support for immobilization, the average increase in the xylitol productivity value was 0.148 g/(L·h) (Table 3). This fact can be related to the highest yield of immobilization resulting from the use of the treated carrier. In this case, the mass transfer rate to the immobilized yeast cells could have enhanced the xylitol production.

The results showed that the treatment of bagasse with hexamethylene diamine aiming to immobilize *C. guilliermondii* resulted in highest adhesion of the cells on support surface. This technique can be useful in immobilizing this yeast to use in repeated-batch or continuous fermentations for xylitol production from hemicellulosic hydrolysates. However, higher mass of carrier in the medium resulted in lower xylitol production during the batch when the cells are adsorbed on the bagasse *in situ* at the beginning of the fermentation. This occurred because, in this case, the cell metabolism deviated the process to the formation of biomass.

Table 3
Effects of Selected Variables on Xylitol Yield ($Y_{p/s}$), Cell Yield ($Y_{x/s}$), Volumetric Xylitol Productivity (Q_p), and Yield of Immobilization (η_{imob})

	$Y_{p/s}$ (g/g)				$Y_{x/s}$ (g/g)				Q_p (g/[L·h])				η_{imob} (%)			
Variable	Effects	SE	*t*	*p*	Effects	SE	*t*	*p*	Effects	SE	*t*	*p*	Effects	SE	*t*	*p*
Mean	0.504	0.013	37.720	0.0000	0.182	0.009	19.515	0.0000	0.374	0.021	18.103	0.0000	18.529	1.242	14.924	0.0000
X_1	–0.098	0.033	–2.991[a]	0.0304[a]	0.120	0.023	5.266[a]	0.0033[a]	–0.050	0.050	–0.992	0.3669	17.795	3.031	5.871[a]	0.0020[a]
X_2	0.034	0.032	1.036	0.3476	–0.011	0.023	–0.479	0.6523	–0.010	0.050	–0.191	0.8559	–4.573	3.011	–1.519	0.1893
X_3	0.059	0.027	2.208	0.0783	–0.034	0.019	–1.801	0.1315	0.148	0.041	3.585[a]	0.0158[a]	9.186	2.483	3.699[a]	0.0140[a]
X_1X_2	–0.048	0.033	–1.457	0.2049	–0.020	0.023	–0.878	0.4203	–0.020	0.050	–0.397	0.7080	–4.500	3.031	–1.485	0.1978
X_1X_3	0.003	0.033	0.077	0.9418	0.015	0.023	0.658	0.5394	0.035	0.050	0.694	0.5185	4.945	3.031	1.631	0.1637
X_2X_3	0.009	0.032	0.284	0.7878	–0.029	0.023	–1.293	0.2524	–0.004	0.050	–0.073	0.9447	–2.183	3.011	–0.725	0.5010

[a]Significance at 95% confidence level.

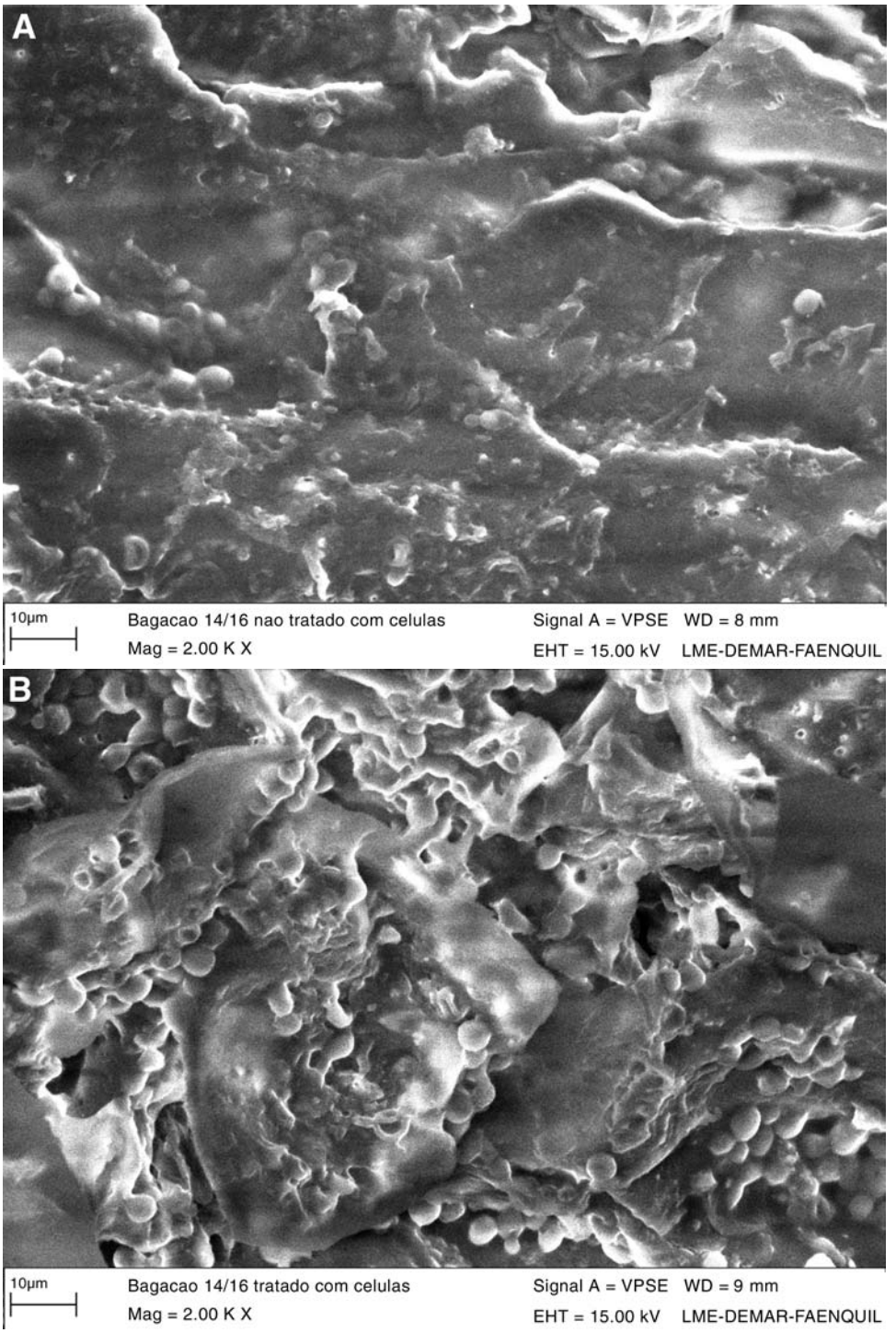

Fig. 2. Micrograph of **(A)** raw sugarcane bagasse and **(B)** treated sugarcane bagasse, obtained after cell adhesion by SEM (average diameter of bagasse: 1.080 mm; magnification: 600-fold).

Conclusion

This work demonstrates that sugarcane bagasse can be used not only as a source of xylose for the bioproduction of xylitol, but also as a carrier for immobilizing the cells. The immobilization procedure is quite simple and can be easily integrated with the bioconversion itself. Activation of the raw bagasse with epichlorohydrin and hexamethylene diamine prior to cell immobilization improved the yield of immobilization and xylitol productivity. The increase in the amount of bagasse used to immobilize the cells led to substantial increases in both cell yield and immobilization yield. Under such conditions, xylitol yield was reduced owing to a higher concentration of suspended solids in the flasks, which favors oxygen dissolution into the medium. The average diameter of the bagasse used to immobilize the cells did not influence significantly any of the response variables evaluated: xylitol yield, cell yield, xylitol productivity, and yield of immobilization.

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