Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-024-05703-1

ORIGINAL ARTICLE

Efficient production of cellooligosaccharides and xylooligosaccharides by combined biological pretreatment and enzymatic hydrolysis process

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Received: 9 February 2024 / Revised: 20 April 2024 / Accepted: 26 April 2024

Abstract

Xylooligosaccharide and cellooligosaccharide production has been improved over the years using different kinds of methods. Chemical and physical methods are the most used due to their facility in reducing the biomass recalcitrance; however, such methods require a great deal of energy and generate undesirable compounds. On the other hand, biological pretreatment is an option to overcome the high energy demand and pollution issues and optimize production. In this work, sugarcane bagasse was biologically pretreated with *Coniophora puteana* (CBMAI 0870), *Gloeophyllum trabeum* (CBMAI 0872), and *Pleurotus ostreatus* (CCIBt 2338) for 5 months. After the biological pretreatment, part of the material was milled in a knife mill (20-mesh) and another part was milled in a ball mill (powder aspect). The material was enzymatic hydrolyzed with cellulase (20, 50, and 100 IU/g) and xylanase (50 IU/g) combined with the milling techniques to produce cellooligosaccharides and xylooligosaccharides. Xylooligosaccharides and cellooligosaccharides were characterized by ATR-FTIR analysis, scanning electron microscopy images and X-ray to evaluate the modifications in the lignocellulosic structure. The enzymatic hydrolysis using cellulase (50 IU/g) combined with knife milling resulted in 26.23% of cellooligosaccharide conversion after 5 months of cultivation with *C. puteana*, while cellulase with the same enzymatic charge combined with ball milling resulted in 36.65% of cellooligosaccharide conversion using the same fungus and the same time of cultivation. Xylooligosaccharide conversion also reached better results when compared to untreated material: the best result was 78.12% of xylooligosaccharide conversion after the biological pretreatment with *G. trabeum*. Finally, scanning electron microscope images made it possible to observe gaps formed by the fungi growth in the biomass in comparison to untreated material; and x-ray analysis showed evidence of the effect of ball milling pretreatment in the biomass, reducing its crystallinity.

Keywords Ball mill · Biomass recalcitrance · Non-digestible oligomers · Prebiotic compounds · Soluble fiber · White-rot fungi

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1 Introduction

In the literature, the process of converting lignocellulosic biomass into value-added products is commonly referred to as lignocellulosic biorefinery. This approach expands potential applications of lignocellulosic materials beyond generating heat energy and ethanol to include the production of by-products and value-added molecules [1–5]. Two value-added molecules are xylooligosaccharides (XOS) and cellooligosaccharides (COS). These molecules comprise xylose and glucose, respectively, and are known to be interesting to human health. XOS is considered healthy due to its capacity to prevent disorders in human nutrition, such as gastrointestinal disorders and infection, obesity control, and

Published online: 21 May 2024

better nutrient absorption [6–8]. XOS also presents various degrees of polymerization, from 2 to 10 xylose units, and the wide range of pH and thermostability makes this sugar a good prebiotic molecule [9, 10]. On the other hand, COS are oligomers formed by β-1,4 linked D-glucose units, with few numbers of glucose units, starting with 2 units, called cellobiose. A characteristic of COS is that the shorter COS (from 3 to 6 glucose units) are water soluble, while longer COS tend to be insoluble. The soluble COS is not digestible by humans and can be prebiotic. It stimulates the growth of healthy bacteria in vitro, such as *Lactobacillus* and *Bifidobacterium* [11–14]. To reach the desirable material, such as COS and XOS, lignocellulosic biomass is submitted to a pretreatment and enzymatic hydrolysis. The pretreatment is important to reduce the recalcitrance of the lignocellulosic material, while the enzymatic hydrolysis will break down the sugars into small units, forming COS and XOS.

Choosing the correct pretreatment is essential to modify the lignocellulosic material structure, as well as the correct enzyme for the enzymatic hydrolysis step. The main pretreatments to overcome the material recalcitrance are chemical, physical, and biological, or a combination of them [15], and each one has its limits and advantages. Physical and chemical pretreatments are faster; however, they demand more energy and generate undesirable compounds, respectively. An example of chemical pretreatment is the acid and alkali pretreatment. A characteristic of an acid pretreatment is to solubilize hemicellulose and lignin, while the alkali pretreatment changes the lignin structure, reducing cellulose crystallinity and partially solubilizing the hemicellulose. An example of physical pretreatment can be steam explosions, which are used to auto-hydrolyze hemicellulose, or ball milling, reducing the size of the lignocellulosic material particles. On the other hand, biological pretreatment is cheaper and does not generate undesirable compounds. However, it requires time and proper isolation without contaminants to the process and depends on the microorganism involved in the process. White rot fungi, for example, are known to degrade lignin, while brown rot fungi degrade cellulose and hemicellulose [15–17].

The tendency to overcome the limitations of the pretreatments is to use a combination of different pretreatments [15, 16]. Various methods could be used to achieve a good COS and XOS yield [9, 18–20], according to each pretreatment characteristic. The inspiration to use a biological pretreatment before enzymatic hydrolysis came from the fungi's capacity of changing the lignocellulosic structure, decreasing the biomass recalcitrance and facilitating the enzymatic hydrolysis [16]. Moreover, using different milling methods is a good alternative to decrease biomass recalcitrance even more [21].

Production of value-added products has been improved over the last decade and different technologies are important to find sustainable ways to produce more with less environmental impact. Experiments using biologically pretreated material followed by enzymatic hydrolysis have a huge potential for eco-friendly production. Regarding this, the objective of this study is to optimize the COS and XOS production using a combination of biological pretreatment combined with physical pretreatments before the enzymatic hydrolysis step, based on the hypothesis that both pretreatments can decrease the sugarcane bagasse recalcitrance. Moreover, an analysis with ATR-FTIR spectra, scanning electron microscope, and an X-ray technique were applied to verify the structural changes in the biomass [16].

2 Materials and methods

2.1 Material and bagasse preparation

The sugarcane bagasse was supplied by a local factory (Usina São João, Araras, SP, Brazil). The industrial bagasse was humidified to 75% and transferred to polypropylene bags. A falcon tube was placed into the central part of each bag as an opening for introducing the fungal inoculum after the set was twice autoclaved at 121 °C for 1 h. The control sample was prepared the same way, however no fungi was inoculated. A total of 15 bags were prepared/fungal strain.

2.2 Biological pretreatment

The microorganisms used in this study were *Coniophora puteana* (CBMAI 0870) and *Gloeophyllum trabeum* (CBMAI 0872) (Brazilian Collection of Environment and Industry Microorganisms, Campinas State University-Unicamp, Campinas, São Paulo, Brazil), and *Pleurotus ostreatus* (CCIBt 2338) (from the São Paulo Botanic Institute).

The microorganisms were cultivated for 7 to 15 days at 25 °C in Potato Dextrose Agar (PDA) (Kasvi Ltda). To prepare the PDA medium, 39 g of PDA was added to 1 L of distilled water; then, it was autoclaved in 200-mL portions. The liquid was poured into Petri dishes (20 mL each); then, it was exposed to UV light. After completing the PDA preparation, the microorganisms were reactivated and put in the Petri dishes [22]. One-fifth of the PDA from Petri dishes was inoculated in the polypropylene bag. Inside the polypropylene bag, each culture was left to grow for 1 to 5 months at 25 °C. Every month, one bag was taken as a sample and the biologically pretreated bagasse was dried at room temperature, milled at 20-mesh, and oven-dried at 60 °C for 24 h. A fraction of the biological pretreated sugarcane bagasse was milled in a ball milling for 1 h for later experiments. The samples were separated according to the fungus applied in the pretreatment.

2.3 Chemical characterization

After the biological pretreatment, and also for the control sample, the sugarcane bagasse chemical characterization (CC) was carried out as follows: 0.3 g of bagasse (dry basis) was added with 3 mL of 72% sulfuric acid (Sigma-Aldrich 320,501-1L) to a 100 mL Schott bottle and allowed to react in a water bath at 45 °C for 7 min. After the reaction time, 84 mL of distilled water was added and then autoclaved for 30 min at 121 °C. Then, a filtration step, using a crucible (16 µm), separated the liquid and the solid fractions. The liquid fraction was used to determine the soluble lignin and the sugars and acetic acid in HPLC, using a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column in 45 °C, a refraction index detector Waters 2414, H_2SO_4 50 mM of mobile phase, a flow of 0.6 mL/min, and a sample volume of 20 µL. The solid fraction was dried for 24 h in an oven at 105 °C to determine the insoluble lignin. The soluble lignin was measured using a spectrophotometer at 215 and 208 nm adopting a standard method [23]. The total lignin was reported, meaning the sum of soluble and insoluble lignin.

2.4 Enzymatic hydrolysis

In the present study, two enzymes were used: cellulase cocktail (Cellic Ctec2–Novozymes, with 1455 IU/mL of endoglucanase) and xylanase. The xylanase was produced from *Aspergillus versicolor* (isolated from Brazilian soil) at our lab [24]. *A. versicolor* was grown on oat wheat and the xylanase was purified using chromatography on DEAE-Sephadex A-50 column (20 × 1.8 cm) equilibrated with the dialysis buffer and Sephadex G-75 column (70 × 1.8 cm) equilibrated with the same buffer [25]. The purified endoxylanase showed activity of 414.4 IU/mL.

For all experiments, the enzymatic hydrolysis was performed with 1 g of material, in 5 mL of sodium acetate buffer solution in pH 5.2. The buffer solution was prepared with sodium acetate (Dinâmica–Química Contemporânea Ltda, COD: 1015) and acetic acid (Dinâmica–Química Contemporânea Ltda, COD: P.10.0021.000.00.81). It was performed for 24 h at 50 °C (for cellulase) or 55 °C (for xylanase) in a shaker at 150 rpm. After hydrolysis, the samples were boiled for 7 min, filtered in 0.44 µm and 0.22 µm filters, and then went through HPLC to determine the sugars. The enzymatic hydrolysis was made in three different steps, differing in the milling method, enzyme, and enzymatic charge. The first experiment was enzymatic hydrolysis with cellulase using material milled in a knife mill (20-mesh) and three different charges: 20 IU/g, 50 IU/g, and 100 IU/g. The second one used cellulase cocktail after milling the material in a ball mill, using an intermediary enzyme charge (50 IU/g). In this experiment, the samples cultivated for 1 and 5 months were hydrolyzed. The ball milling was performed in a 150 mL reactor using a 110 g metallic ball with 617 blows per minute, in a ball mill model MA 350 (MARCONI Equipamentos para Laboratórios Ltda). In the third experiment, the samples were milled using a knife mill, and the hydrolysis was performed using xylanase with an intermediary enzyme charge (50 IU/g).

2.5 Analysis of ATR-FTIR

Knife-milled untreated and biologically pretreated bagasse before and after enzymatic hydrolysis with cellulase with 50 IU/g were analyzed by infrared (FTIR) attenuated total reflectance (ATR), using an FTIR-IRAffinity-1S Shimadzu spectrophotometer. The samples analyzed by FTIR-ATR were only from the first and last months of cultivation as the objective of this experiment was to study the difference caused by the biological pretreatment and enzymatic hydrolysis in the lignocellulosic structure.

2.6 Biomass images from electron microscope

The 20-mesh milled untreated and biologically pretreated samples of three consecutive months were measured using scanning electron microscopy (SEM, EVO-LS-15, Carl Zeiss), operated at 15 kV with a spot size of 3.0–4.0 mm and a working distance (WD) of 9.5 mm.

2.7 X-ray analysis

The analyzed samples were from the first and last months of cultivation for each microorganism and also with untreated material. All samples were compared after grinding in the ball mill and knife mill with a particle size of 20 mesh. A Shimadzu XRD-6000 model diffractometer equipped with a Cu anode ($\lambda = 1.54059$ Å) was used. Measurements were taken at 40 kV and 30 mA voltages in θ–2θ configuration from $2\theta = 13$ to 25°, with a step size of 0.02° and a scan speed of 2°/min. The proportion of crystalline regions in bioplastics was determined from the crystallinity index (CrI), from Eq. 1:

$$\mathrm{CrI} = \left[\frac{\mathrm{I20} - \mathrm{Iam}}{\mathrm{I20}}\right] * 100$$

Equation 1: Crystallinity index formula

where I20 is the intensity from plane diffraction at $2\theta = 20°$, and Iam represents the background scattering intensity [26].

2.8 Statistical analysis

The statistical analysis was made in DX3Trial-expert software (DesignExpert, Stat-Ease company, version DX 6.0.6). ANOVA was used to compare statistical differences between

untreated material with the biologically pretreated material. Different letters mean statistical differences between the samples.

3 Results and discussion

3.1 Chemical characterization

The sugarcane bagasse was submitted to a chemical characterization to determine the percentage of cellulose, hemicellulose, and lignin (Table 1). Corroborating previous literature [16], the changes in the lignocellulosic components can be observed. It is important to note that, in the chemical composition table, the ANOVA test was made comparing only the untreated samples to the samples corresponding to each microorganism, not comparing the microorganisms themselves (Supplementary Table 1).

A consequence of biological pretreatment is the consumption of cell wall components. The implication of consuming the cellulose and hemicellulose was the lignin content increase after the biological pretreatment. In this case, the lignin content of the samples pretreated with *G. trabeum* and *C. puteana* reached 60.49% after 1 month of cultivation with *G. trabeum*, while the untreated samples reached 21.55% of lignin content. In this case, both fungi statistically changed the lignin content of the biomass compared to untreated samples. However, the lignin content of untreated samples was statistically similar for *P. ostreatus*, except for the fourth month, reaching 34.60% of lignin content. The increase in lignin is related to the lignocellulosic structure modifications as fungi tend to first consume more readily available sugars instead of lignin [27].

For cellulose content, almost all samples were statistically similar to the untreated sample, except for the one that was biologically pretreated with *C. puteana* after 5 months. The higher cellulose content was observed after 2 months of biological pretreatment with *G. trabeum*, reaching 45.40%, while the lower was in the last month of cultivation with *C. puteana* 30.69%. For total hemicelluloses, a decrease for *G. trabeum* and *C. puteana* was observed, from 27.66% for untreated samples to 16.7% after 1 month of cultivation with *G. trabeum*, and 15.17% for *C. puteana* after 4 months of biological pretreatment. However, the hemicelluloses percentage increased after 5 months of cultivation with *P. ostreatus*, reaching 38.86%. Generally, the hemicellulose content changed statistically in almost all samples, except those from 1-month culture with *C. puteana* and *P. ostreatus*. The same pattern for cellulose and hemicellulose content after biological pretreatment with these fungi was reported in the literature [16].

The preference for sugars or lignin is related to the microorganism enzymatic arsenal. White-rot fungi prefer to degrade lignin, while brown-rot fungi degrade other polysaccharides. However, regardless of which macromolecule was consumed, a consequence of degradation is the change in the lignocellulosic structure and its recalcitrance [28]. Once the recalcitrance decreases, the enzymatic hydrolysis may be more efficient in COS and XOS production, which

Table 1 Chemical composition of untreated and biologically pretreated sugarcane bagasse material

Microorganism	Growth time (months)	Cellulose (%)	Hemicellulose (%)	Total lignin (%)
Untreated	**0**	$40.53 \pm 5.41^{a,b}$	27.66 ± 3.15^{a}	21.55 ± 0.20^{a}
Gloeophyllum trabeum (CBMAI 0872)	**1**	37.60 ± 1.63^{a}	16.7 ± 1.74^{b}	60.49 ± 1.70^{b}
	2	45.40 ± 0.96^{b}	20.08 ± 2.09^{b}	53.06 ± 4.62^{b}
	3	37.14 ± 1.07^{a}	17.57 ± 4.72^{b}	$33.57 \pm 1.65^{a,d}$
	4	$42.42 \pm 2.94^{a,b}$	16.06 ± 1.75^{b}	57.70 ± 1.41^{b}
	5	$43.30 \pm 1.16^{a,b}$	15.66 ± 0.25^{b}	34.95 ± 3.91^{d}
Coniophora puteana (CBMAI 0870)	**1**	41.55 ± 0^{a}	$22.12 \pm 5.44^{a,c}$	$25.24 \pm 0.38^{a,c}$
	2	43.27 ± 0.06^{a}	$16.22 \pm 0.08^{b,c}$	41.58 ± 0.77^{b}
	3	41.21 ± 1.53^{a}	$17.98 \pm 0.46^{b,c}$	$38.09 \pm 7.91^{b,c}$
	4	32.81 ± 1.72^{c}	15.17 ± 0.11^{b}	42.75 ± 2.74^{b}
	5	30.69 ± 0.00^{c}	15.24 ± 0.19^{b}	$36.63 \pm 1.86^{b,c}$
Pleurotus ostreatus (CCIBt 2338)	**1**	35.84 ± 0.54^{a}	$32.33 \pm 3.58^{a,c}$	$26,43 \pm 0^{a,b}$
	2	40.12 ± 0.06^{a}	$35.79 \pm 3.85^{b,c}$	$29.72 \pm 4.86^{a,b}$
	3	39.51 ± 0.36^{a}	$38.35 \pm 1.74^{b,c}$	$26.07 \pm 2.19^{a,b}$
	4	41.29 ± 0.92^{a}	$38.34 \pm 0.63^{b,c}$	34.60 ± 3.17^{b}
	5	40.28 ± 1.00^{a}	38.86 ± 0.80^{b}	$26.47 \pm 0.52^{a,b}$

Significant statistical differences (p-value less than 0.05) were symbolized by different letters in the same column for each microorganism

is the main objective of this work, leading this characteristic to have a major impact on the final results.

3.2 Enzymatic hydrolysis

The enzymatic hydrolysis occurred both in untreated and biologically pretreated sugarcane bagasse samples using three different enzymatic charges, including ball and knife mills. The purpose of differing the enzymatic charges was to compare the effectiveness of enzyme charge over the 20-mesh milled bagasse, after the biological pretreatment. Besides that, a comparison using the material after ball milling was made. The statistical analysis compared the untreated samples with each microorganism, and then, the enzymatic charges for each line of the table.

Table 2 presents the bagasse milled at 20-mesh, hydrolyzed by cellulase at 20, 50, and 100 IU/g. The table that represents the quantity of glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, and larger than cellohexaose, individually, can be found in Supplementary Table 2, in the Supplementary material. For most samples, the COS yield was better using 100 IU/g of cellulase. However, the most evident value was 26.39% of COS yield in the fifth month of biological pretreatment with *Coniophora puteana* using 50 IU/g of enzyme charge. Comparing the data from the COS yield, it was observed by using the ANOVA test that most of the results between 50 and 100 IU/g were not significantly different.

The material treated with *G. trabeum* and enzymatic hydrolysis produced more COS than the untreated one; however, most samples were statistically similar, except those cultivated for 5 months (20 IU/g), reaching 14.47% of COS; 1, 3, and 5 months (50 IU/g), reaching 12.37%, 12.76%, and 11.38% of COS, respectively; and 1 month (100 IU/g), reaching 16.91% of COS. *C. puteana* followed the same pattern as *G. trabeum*. In this case, the samples that are different were cultivated for 5 months (20 IU/g and 100 IU/g) and 4 and 5 months (50 IU/g). A different pattern was shown by *P. ostreatus* when enzymatic hydrolyzed with cellulose 50 and 100 IU/g were used: most of the results presented less COS yield compared to the untreated biomass, reaching only 3.52% of COS yield using 50 IU/g of cellulase in the material cultivated for two months.

Considering the comparison of COS yield among the fungi, it is important to highlight that some samples showed significant differences throughout all the months, such as the fifth month for *G. trabeum* and the third month for *P. ostreatus* when using 20 IU/g; and the fifth month for *C. puteana* using 50 and 100 IU/g of cellulase. All ANOVA tests for enzymatic hydrolysis are shown in the Supplementary material (Tables 3, 4 and 5).

Table 3 shows the COS yield (%) of cellulase applied to biologically pretreated sugarcane bagasse after ball milling, including the quantities of glucose, celobiose, cellotriose, celotetraose, cellopentaose, cellohexaose, and sugars larger than cellohexaose, individually, in the same conditions. In this experiment, only 2 months of cultivation were selected.

Table 2 COS yield (%) after enzymatic hydrolysis of biologically pretreated sugarcane bagasse using three different enzymatic charges of cellulase

Samples (20-mesh milled)	Cultivation time (months)	Enzymatic charge		
		20 IU/g	50 IU/g	100 IU/g
Untreated	-	2.42 ± 2.57^{aA}	5.65 ± 1.56^{aA}	9.39 ± 4.15^{aA}
Gloeophyllum trabeum (CBMAI 0872)	1	4.61 ± 0.64^{aA}	$12.37 \pm 0.04^{bA,B}$	16.91 ± 5.33^{bB}
	2	4.10 ± 2.51^{aA}	7.04 ± 2.12^{aA}	$10.11 \pm 0.83^{a,bA}$
	3	6.52 ± 2.50^{aA}	12.76 ± 2.10^{bB}	$14.97 \pm 0.08^{a,bB}$
	4	5.96 ± 1.55^{aA}	$8.08 \pm 2.11^{a,cA}$	9.17 ± 0.66^{aA}
	5	14.47 ± 1.63^{bA}	$11.38 \pm 0.62^{b,cA}$	$11.51 \pm 3.98^{a,bA}$
Coniophora puteana (CBMAI 0870)	1	2.30 ± 0^{aA}	4.87 ± 0.96^{aA}	6.58 ± 2.69^{aA}
	2	4.70 ± 0^{aA}	$9.77 \pm 0.88^{a,cA}$	10.86 ± 3.58^{aA}
	3	5.79 ± 1.58^{aA}	$9.01 \pm 2.98^{a,cA,B}$	13.22 ± 0^{aB}
	4	10.07 ± 2.08^{aA}	$12.46 \pm 0.81^{b,cA}$	11.78 ± 1.96^{aA}
	5	21.79 ± 7.09^{bA}	26.39 ± 3.29^{dA}	25.22 ± 7.29^{bA}
Pleurotus ostreatus (CCIBt 2338)	1	$5.24 \pm 0^{a,bA}$	5.93 ± 0.13^{aA}	$6.21 \pm 1.30^{a,bA}$
	2	$5.61 \pm 0^{a,bA}$	3.52 ± 1.39^{aA}	$5.60 \pm 0.75^{a,bA}$
	3	7.80 ± 1.14^{bA}	4.69 ± 1.14^{aB}	$4.77 \pm 0^{bA,B}$
	4	4.09 ± 1.29^{aA}	6.40 ± 1.72^{aA}	$6.32 \pm 0.62^{a,bA}$
	5	$5.24 \pm 0.71^{a,bA}$	4.68 ± 1.03^{aA}	4.53 ± 1.10^{bA}

Significant statistical differences (p-value lesser than 0.05) were symbolized by different letters in the same column and line for each microorganism

Table 3 COS yield (%) after enzymatic hydrolysis of ball-milled biologically pretreated sugarcane bagasse using cellulase

Samples (milled with ball-mill)	Cultivation time	Enzymatic charge of 50 IU/g							
Fungi	Month	Glucose	C2	C3	C4	C5	C6	> C6	COS
Untreated		33.24	7.21	0	0.1	0	1.58	0	8.87 ± 0.28^{a}
G. trabeum	1	46.26	15.28	1.29	0.1	1.48	4.44	0	22.58 ± 1.64^{b}
	5	30.28	5.24	0	0.44	0.39	3.50	0	9.56 ± 0.36^{a}
C. puteana	2	32.55	10.12	0.98	0.09	1.11	3.35	0.01	15.64 ± 1.34^{b}
	5	64.13	22.83	1.97	0.13	1.6	10.12	0.01	36.65 ± 2.52^{c}
P. ostreatus	1	38.83	6.66	0	0.11	0	2.10	0	8.86 ± 0.87^{a}
	4	48.58	11.39	0.69	0.25	0.72	3.08	0	16.11 ± 1.11^{b}

Significant statistical differences (p-value lesser than 0.05) were symbolized by different letters in the same column for each microorganism

C1 glucose, *C2* celobiose, *C3* celotriose, *C4* celotetraose, *C5* celopentaose, *C6* celohexaose, > C6 chains larger than celohexaose, *COS* gluco-oligosaccharides = C2 + C3 + C4 + C5 + C6 + > C6

The untreated sample showed the lowest COS yield (8.87%). For the biologically pretreated material with *G. trabeum,* the best result was 22.58%, cultivated for 1 month. For *Coniophora puteana* and *Pleurotus ostreatus*, the best result was in the fifth (36.65%) and fourth month (16.11%) of cultivation, respectively. The value of 36.65% of COS yield for *C. puteana* was the best result for this experiment: more than twice the COS conversion compared to the sample corresponding to the first month. The ANOVA analysis showed the statistical comparison between the untreated sample and each microorganism. The statistical similarity appeared only for the samples represented by month 5 for *G. trabeum* and month 1 for *P. ostreatus*. All samples compared to each other showed significant differences (Supplementary material).

The result of COS yield (Table 3), where all samples were milled with ball milling, was greater than any other sample milled to the 20-mesh, including those that used 100 IU/g of cellulase in the enzymatic hydrolysis. Ball milling was already used as an enhancer for enzymatic hydrolysis of cellulosic materials. Mais et al. (2002) [29] used ball milling to increase the cellulose hydrolysis in *Pseudotsuga menziesii* (Pinophyta, Plantae) both at room temperature (25 °C) and at 45 °C. In this case, pre- and post-treatments in the lignin removal were reported. Wu et al. (2021) [30] used ball milling to enhance enzymatic hydrolysis in *Populus tremuloides* (Magnoliophyta, Plantae) and reported 66.5% of glucose at a low enzyme dose (10 FPU/g). The difference in this work came from the milling step: the samples were intermittently milled to optimize the enzymatic hydrolysis, while in the present work, the milling and hydrolyzation were carried out in different steps. In addition, the ball milling pretreatment modified the microcrystalline cellulose properties, managing to increase the capacity enzymatic hydrolysis [21]. Although those experiments did not aim to produce COS, the effect of ball milling used together with enzymatic hydrolysis was clear: the ball milling method enhanced the capacity of enzymatic hydrolysis. In fact, the combination of ball milling with other methods to enhance enzymatic processes or production of some biomass-based products are common in the literature [31–35]. However, there is a lack of literature on experiments using ball milling and biological pretreatment to enhance enzymatic hydrolysis for COS production. The results of the present study show the great potential of this technique, and ball milling after biological pretreatment can be used together for other purposes.

XOS production was evaluated using xylanase (50 IU/g) applied to 20-mesh untreated and biologically pretreated material (Table 4). The untreated sample reached 2.95% of XOS yield. For *G. trabeum* and *C. puteana*, almost all samples converted more than 40% of XOS, reaching 78.56% in the sample pretreated with *G. trabeum* after one month of cultivation. For the samples pretreated with *C. puteana*, the best result was achieved after 5 months of cultivation, reaching 66.26% of XOS yield. The samples that were biologically pretreated with *P. ostreatus* had the lowest XOS conversion of XOS in the first month of cultivation, 9.17%. For xylanase production, the statistical comparison revealed significant differences between all samples compared to the untreated one.

The XOS production occurred through the breakdown of the glycosidic linkage in the xylan chain. For XOS production, there are a few studies in the literature using a milling technique and a biological pretreatment together [36]. These are usually performed using chemical, physical–chemical pretreatments, or enzymatic hydrolysis, aiming at the food, medical and pharmaceutical industries [37]. Experiments producing XOS reached 67.43% of XOS conversion using *Aspergillus versicolor* endoxylanase with

Table 4 XOS production after enzymatic hydrolysis of untreated and biologically pretreated sugarcane bagasse

Samples (20-mesh)	Cultivation time	Enzymatic charge 50 IU/g						
XOS (%)								
Fungi	Month	X2	X3	X4	X5	X6	> X6	XOS
Untreated		0	0	0.06	0	0	2.90	2.95 ± 0.10^{a}
G. trabeum	1	21.41	9.68	0.12	0	0	47.35	78.56 ± 0.03^{b}
	2	16.39	6.55	0.08	0	0	31.74	54.75 ± 0.43^{c}
	3	0	3.63	0.08	0.07	0	38.22	41.99 ± 5.92^{d}
	4	0	3.56	0.11	0	0	3845	42.12 ± 6.18^{d}
	5	0	10.96	0.11	0	0	39.02	$50.08 \pm 5.61^{c,d}$
C. puteana	1	0	0	0.08	0	4.86	10.37	15.31 ± 0.01^{b}
	2	0	9.39	1.19	1.34	0	39.13	51.05 ± 1.02^{c}
	3	0	6.80	1.72	0	0	36.63	45.15 ± 1.20^{d}
	4	0	6.71	0.50	0	0	41.58	48.78 ± 1.82^{d}
	5	0	4.43	0.11	0	0	61.73	66.26 ± 10.41^{c}
P. ostreatus	1	0	0	0.05	0	0	9.13	9.17 ± 0.001^{b}
	2	0	0	0.04	0	0	7.40	7.44 ± 0.03^{c}
	3	0	0	0.04	0	0.92	7.77	8.72 ± 0.44^{b}
	4	0	0	0.04	0	0	9.05	9.09 ± 0.46^{b}
	5	0	0	0.04	0	0.76	6.94	7.73 ± 0.44^{c}

Significant statistical differences (p-value lesser than 0.05) were symbolized by different letters in the same column for each microorganism

*X1 xyl*ose, *X2* xylobiose, *X3* xylotriose, *X4* xylotetraose, *X5* xylopentaose, *X6* xylohexaose, > X6 chains larger than xylohexaose, *XOS* Xylo-oligosaccharides = X2 + X3 + X4 + X5 + X6 + > X6

a 65 IU/g of enzymatic load for 24 h at 55 °C in sugarcane bagasse and leaves [3], while this experiment reached improved results (78.12% after 1 month of cultivation with *Gloeophyllum trabeum*) using less enzyme. However, in this case, the enzyme was used directly in the xylan, which can optimize the results. It was observed that the quantity of xylose varied according to the microorganism and time of cultivation. It is known that the reaction time, temperature, quantity and type of enzyme and even the rotation/shaking (rpm) during enzymatic hydrolysis can change the xylose and XOS yield [38].

3.3 FTIR-ATR analysis

FTIR-ATR was applied to untreated and biologically pretreated material in different cultivation times and before and after enzymatic hydrolysis using cellulase with 50 IU/g of enzymatic charge (Fig. 1). All samples were milled in a 20-mesh. Table 5 presents wavebands corresponding to the functional groups in sugarcane bagasse.

Both samples of *C. puteana* and *P. ostreatus* showed bands at 1750–1500 cm^{-1} region, after enzymatic hydrolysis. It is known that this region corresponds to the

Table 5 FTIR-ATR absorbance wavenumber in lignocellulosic biomass

Wavenumber (cm^{-1})	Functional group	Polymer	Reference
1735	C = O stretching	Hemicellulose	[39]
	Carbonyl-ester bond stretching of the p-coumaric acid unit	Lignin	[40]
1600	Aromatic ring vibration	Lignin	[41]
1510	C=C in aromatic ring	Lignin	[41]
1245	C–O of the acetyl group	Hemicellulose	[42, 43]
1161	C–O–C vibration pyranose ring	Cellulose, hemicellulose	[44, 45]
1050	C–O–C vibrations in the anomeric region of the xylans	Hemicellulose	[46, 47]
897	C–O–C stretch of β-glycosidic bonds	Cellulose, hemicellulose	[48–50]
833	C–H out-of-plane in p-hydroxyphenyl units	Lignin	[51]

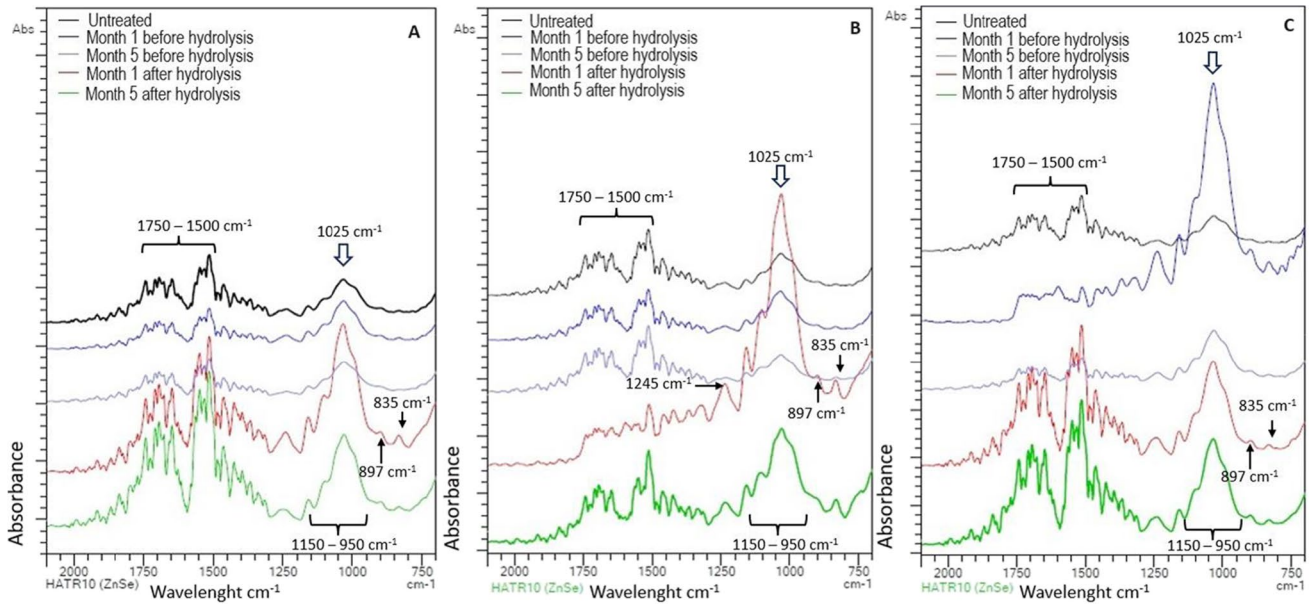

Fig. 1 **A** FTIR-ATR spectra of untreated and biologically pretreated with *C. puteana* (CBMAI 0870) bagasse samples. **B** FTIR-ATR spectra of untreated and biologically pretreated with *G. trabeum* (CBMAI 0872) bagasse samples. **C** FTIR-ATR spectra of untreated and biologically pretreated with *P. ostreatus* (CCIBt 2338) bagasse samples

non-conjugating C = O stretching functional group from hemicellulose and C = C stretching from the aromatic ring vibration from lignin, respectively [52]. The bands around 1510 cm^{-1} are evident in all samples after enzymatic hydrolysis, showing a modification in the lignin structure after the enzymatic hydrolysis with cellulase, once lignin and the sugars are strictly connected in the plant cell wall. The band 835 cm^{-1} also is atributed to lignin (C-H out of plane in *p*-hydroxyphenyl units) and it is evident in all samples after enzymatic hydrolysis [51, 53].

All enzymatically hydrolyzed samples also showed a band at the 1025 cm^{-1} wavelength. This band is between a region (897 and 1159 cm^{-1}) that indicates the presence of hemicellulose and cellulose in the material, specifically the C–O–C β-glycosidic linkages between the sugar units in cellulose and hemicellulose [48, 54]. This showed a change in the polysaccharide structure, specifically a glycosidic linkage formation [55]. For the *C. puteana* and *G. trabeum* samples, in the first month after enzymatic hydrolysis, the clearest peaks in this region were observed, probably due to the effects from the enzyme in the sugars. However, these bands are less evident in the fifth month possibly due to less quantity of sugars at this time of cultivation. This argument can be supported by the quantity of hemicellulose remaining after biological pretreatment for these two fungi (Table 1). This region also covers the 897 cm^{-1} band, which is attributed to vibrations belonging to polysaccharides, which is also evident in all samples after enzymatic hydrolysis.

All samples not enzymatically hydrolyzed showed the same patterns as the untreated sample, except the 1025 cm^{-1} band in the *P. ostreatus*. In this case, the structure of polysaccharides may experience modifications by the microorganism itself. The similarity of the material before enzymatic hydrolysis with untreated material demonstrates that the main modification in the lignocellulosic structure was made only after the enzymatic hydrolysis.

3.4 Scanning electron microscope images

Scanning electron microscopy was applied to compare the degree of physical modification between untreated and biologically pretreated sugarcane bagasse (Fig. 2). Then, 3 months were analyzed in sequence compared to the untreated biomass to show the continuous modification caused by the fungus action. The arrows show gaps formed by the fungi growing, in addition to the continuous degradation during the experiment.

The untreated sugarcane bagasse showed no gaps and a preserved structure. It was observed that the biomass degradation by the fungi started in the cell wall, where it experienced structural modification, changing its recalcitrance and chemical composition [55]. In the cell wall, the components (lignin, cellulose, and hemicellulose) are degraded by specific enzymes produced by the fungus, which can be more or less specific for each macromolecule. Each microorganism has its own enzymatic arsenal, with particular ways of degrading biomass. Some white rot fungi do not completely degrade the lignin complex to reach the polysaccharides, as only a partial

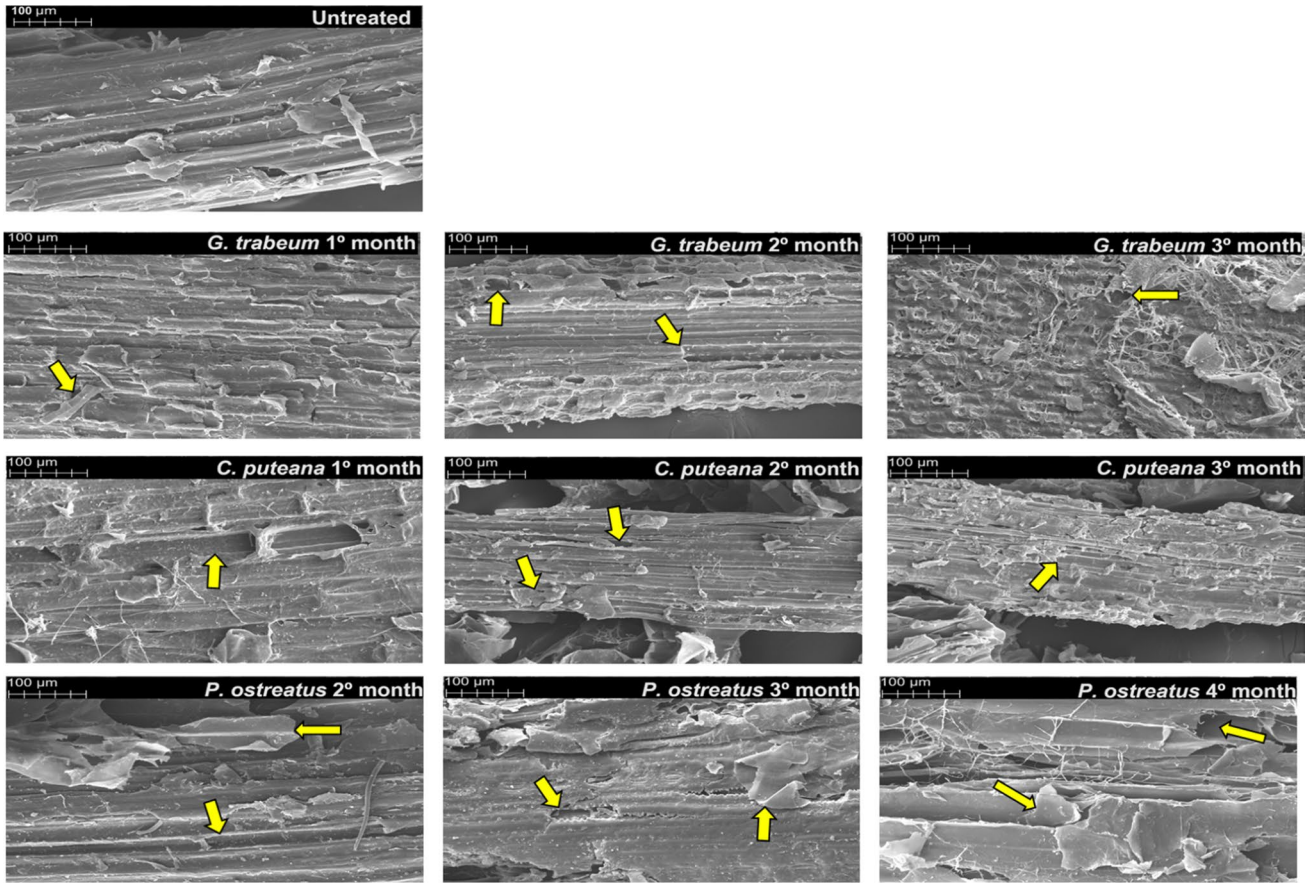

Fig. 2 Scanning electron microscope image from the untreated and biologically pretreated sugarcane bagasse. The yellow arrows show the gaps formed by the fungi growth (*G. trabeum* 2nd month, *G. trabeum* 3rd month, *C. puteana* 1st month, *C. puteana* 2nd month, *P. ostreatus* 2nd month, *P. ostreatus* 3rd month and *P. ostreatus* 4th month) and the structural modifications in the lignocellulosic biomass (*G. trabeum* 1st month, *C. puteana* 2nd month, *C. puteana* 3rd month, *P. ostreatus* 2nd month, *P. ostreatus* 3rd month, and *P. ostreatus* 4th month)

structural modification in the cell wall may allow access of larger molecular size enzymes to the polysaccharides [56].

The biomass degradation by *G. trabeum*, *C. puteana*, and *P. ostreatus* can be seen in Fig. 2, mainly when comparing the beginning and the end of the fungal growth. These images clearly show the way microorganisms modify the lignocellulosic structure to access the cellulose and hemicellulose during the degradation process. The arrows show evidence of the structural modification in the cell wall, while the untreated biomass remains intact.

3.5 X-ray analysis

X-ray diffraction was applied to observe the crystallinity in the lignocellulosic structure, comparing the knife and ball milling (Fig. 3). The X-ray analysis diffraction reveals the peak, meaning the samples have a crystallinity index [26].

Two peaks were observed in all samples using knife milling: the first one around $2\theta = 16°$ and the second one around $2\theta = 22°$. Seiwert et al. (2021) [57] reported that the diffraction pattern in 18.2° is related to the organized xylan, which may be the case at the peak at $2\theta = 16°$. The diffraction pattern at 21–22° is related to the cellulose organized structure at ~ 22.8° [58]. Comparing the curve related to the ball-milled samples, it was not possible to determine the crystallinity, including the untreated ones. In this case, the peak was no longer in $2\theta = 22°$. This characteristic of the curve means low crystallinity of the cellulose chain, meaning that the ball milling method was, in fact, efficient in deconstructing the lignocellulosic structure. This pattern was shown by all samples.

When comparing the results between the crystallinity index and COS production (Table 3), both after ball milling, it can be assumed that this pretreatment is related to the recalcitrance reduction of the biomass. The effect of ball milling was already analyzed in other biomasses for different reasons [59, 60]; however, all the conclusions led to the crystallinity decrease after ball milling.

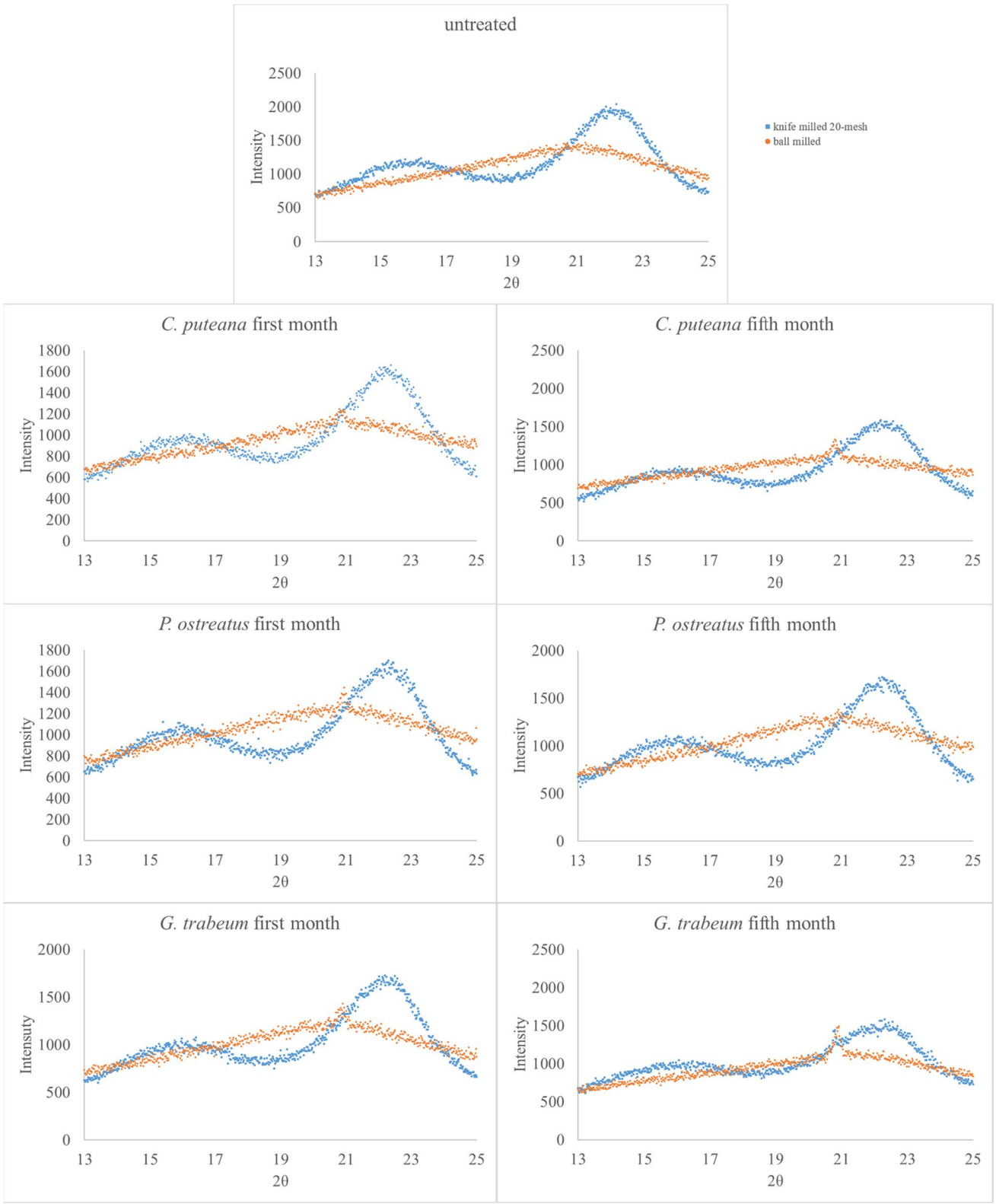

Fig. 3 X-ray diffraction from sugarcane bagasse after biological pretreatment and ball mill

4 Conclusions

This work was based on the hypothesis that biological pretreatment combined with physical methods could modify the lignocellulosic structure. These modifications may reduce the biomass recalcitrance, facilitating the enzymatic hydrolysis step. The biological pretreatment effect was perceptible by the scanning electron images, and the chemical characterization analysis verified that the polysaccharides were partially consumed. However, the COS and XOS yield was better than the untreated biomass, resulting in 26.39% of COS for *C. puteana* after 5 months of cultivation. This occurred due to the effects of fungi growth in the sugarcane bagasse, reducing its recalcitrance. The cellulose crystallinity was reduced after ball milling, as indicated by x-ray analysis. The quantity of COS yield after this pretreatment was higher than any samples that were only milled with a knife mill, reaching 36.65% of COS yield. In fact, after ball milling, only the intermediary enzyme charge (50 IU/g) was sufficient to achieve all values for the COS yield concerning the samples that were knife milled, proving that the ball mill method also reduces the biomass recalcitrance. The higher values for XOS yield were achieved for the samples biologically pretreated with *G. trabeum* and *C. puteana*, reaching 78.56% of XOS for the first one after one month of cultivation. It is important to note that these samples were only milled with the knife mill and the enzyme charge was 50 IU/g. These results can be optimized by increasing the quantity of enzymes or by milling the material in the ball mill. The most evident limitation of the biological pretreatment is the time of cultivation. However, the present study showed potential for combined pretreatments, mainly the biological pretreatments and ball mill method. Future experiments can be conducted using chemical pretreatments or different kinds of milling techniques together with biological pretreatments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13399-024-05703-1.

Acknowledgements The authors would also like to thank E. J. de Souza for the assistance in the SEM analyses.

Funding The authors would like to thank the São Paulo Research Foundation FAPESP (grant no. 2022/03219–2; 2017/22401–8) and the National Council for Scientific and Technological Development CNPQ (grant no. 423730/2021–5; 310846/2022–6; 303239/2021–2).

Data availability Not applicable.

References

1. Sriariyanun M, Dharmalingam B (2023) From waste to wealth: challenges in producing value-added biochemicals from lignocellulose biorefinery. J Appl Sci Emerg Technol 22(3):e900001
2. Ruensodsai T, Sriariyanun M (2022) Sustainable development and progress of lignocellulose conversion to platform chemicals. J KMUTNB 32(4):815–818
3. Forsan CF et al (2023) Xylooligosaccharides production from sugarcane bagasse and leaf using Aspergillus versicolor endoxylanase and diluted acid. Biomass Convers Biorefinary 13:3375–3390. https://doi.org/10.1007/s13399-021-01403-2
4. Scapini T et al (2021) Hydrothermal pretreatment of lignocellulosic biomass for hemicellulose recovery. Biores Technol 342:126033. https://doi.org/10.1016/j.biortech.2021.126033
5. Qaseem MF, Shaheen H, Wu AM (2021) Cell wall hemicellulose for sustainable industrial utilization. Renew Sustain Energy Rev 144:110996. https://doi.org/10.1016/j.rser.2021.110996
6. Adebola OO, Corcoran O, Morgan WA (2014) Synbiotics: the impact of potential prebiotics inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. J Funct Foods 10:75–84. https://doi.org/10.1016/j.jff.2014.05.010
7. Vázquez MJ et al (2000) Xylooligosaccharides: manufacture and applications. Trends Food Sci Technol 11:387–393. https://doi.org/10.1016/S0924-2244(01)00031-0
8. Cao R et al (2020) Investigation on decolorization kinetics and thermodynamics of lignocellulosic xylooligosaccharides by highly selective adsorption with Amberlite XAD-16N. Food Chem 310:125934. https://doi.org/10.1016/j.foodchem.2019.125934
9. Kumar V et al (2021) Developing a sustainable bioprocess for the cleaner production of xylooligosaccharides: an approach towards lignocellulosic waste management. J Clean Prod 316:128332. https://doi.org/10.1016/j.jclepro.2021.128332
10. Mano MCR et al (2017) Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. Appl Microbiol Biotechnol 102:17–37. https://doi.org/10.1007/s00253-017-8564-2
11. Ávila PF et al (2021) Cello-oligosaccharides production from lignocellulosic biomass and their emerging prebiotic applications. World J Microbiol Biotechnol 37:1–11. https://doi.org/10.1007/s11274-021-03041-2
12. Fujii S et al (2016) Combination of soy isoflavones and cellooligosaccharides changes equol/ O -desmethylangolensin production ratio and attenuates bone fragility in ovariectomized mice. Biosci Biotechnol Biochem 80(8):1632–1635. https://doi.org/10.1080/09168451.2016.1184559
13. Karnaouri A et al (2019) Valorization of waste forest biomass toward the production of cello-oligosaccharides with potential prebiotic activity by utilizing customized enzyme cocktails. Biotechnology Biofuels 12(1):1–19. https://doi.org/10.1186/s13068-019-1628-z
14. Sanz ML, Gibson GR, Rastall RA (2005) Influence of disaccharide structure on prebiotic selectivity in vitro. J Agric Food Chem 53(13):5192–5199. https://doi.org/10.1021/jf050276w
15. Felipuci JP et al (2020) Biotechnological aspects of microbial pretreatment of lignocellulosic biomass. In: Verma P (ed) Biorefineries: as step towards reneable and clean energy. Springer, Singapore, pp 121–150. https://doi.org/10.1007/978-981-15-9593-6_6
16. Felipuci JP et al (2021) Biological pretreatment improved subsequent xylan chemical solubilization. Biomass Convers Biorefinery 13:5317–5324. https://doi.org/10.1007/s13399-021-01506-w
17. Rastogi M, Shrivastava S (2017) Recent advances in second generation bioethanol production: an insight to pretreatment, saccharification and fermentation processes. Renew Sustain Energy Rev 80:330–340. https://doi.org/10.1016/j.rser.2017.05.225
18. Santana MB et al (2023) Hydrothermal pretreatment for the production of prebiotic oligosaccharides from tobacco stem. Biores Technol 382:129169
19. Fang L et al (2022) Co-production of xylooligosaccharides and glucose from birch sawdust by hot water pretreatment and enzymatic hydrolysis. Biores Technol 348:126795

20. Mensah RQ et al (2024) Two-step physico-biological production of xylooligosaccharides from sugarcane leaves. Biocatal Agric Biotechnol 55:102962
21. Gomide FTF et al (2019) Modification of microcrystalline cellulose structural properties by ball-milling and ionic liquid treatments and their correlation to enzymatic hydrolysis rate and yield. Cellulose 26:7323–7335
22. Castellani A (1963) Further researches on the long viability and growth of many pathogenic fungi and some bacteria in sterile distilled water. Mycopathologia 20(1–2):1–6
23. Gouveia ER et al (2009) Validation of methodology for the chemical characterization of sugar cane bagasse. Quim Nova 32:1500–1503. https://doi.org/10.1590/S0100-40422009000600026
24. Freitas C et al (2021) In vitro study of the effect of xylooligosaccharides obtained from banana pseudostem xylan by enzymatic hydrolysis on probiotic bacteria. Biocatal Agric Biotechnol 33:101973. https://doi.org/10.1016/j.bcab.2021.101973
25. Carmona E et al (1998) Purification and biochemical characterization of an endoxylanase from Aspergillus versicolor. FEMS Microbiol Lett 66(2):311–315. https://doi.org/10.1111/j.1574-6968.1998.tb13906.x
26. Macedo JVC et al (2022) Xylan-starch-based bioplastic formulation and xylan influence on the physicochemical and biodegradability properties. Polym Bull 80:8067–8092. https://doi.org/10.1007/s00289-022-04385-x
27. Kerem Z, Jensen KA, Hammel KE (1999) Biodegradative mechanism of the brown rot basidiomycete Gloeophyllum trabeum: evidence for an extracellular hydroquinone-driven fenton reaction. FEBS Lett 446(1):49–54. https://doi.org/10.1016/S0014-5793(99)00180-5
28. Zeng J, Singh D, Gao D, Chen S (2014) Effects of lignin modification on wheat straw cell wall deconstruction by Phanerochaete chrysosporium. Biotechnol Biofuels 7(161):1–13. https://doi.org/10.1186/s13068-014-0161-3
29. Mais U et al (2002) Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling. In: Finkelstein M, McMillan JD, Davison BH (eds) Biotechnology for fuels and chemicals. Applied Biochemistry and Biotechnology. Humana Press, Totowa, NJ, pp 815–832. https://doi.org/10.1007/978-1-4612-0119-9_66
30. Wu Y et al (2021) Application of intermittent ball milling to enzymatic hydrolysis for efficient conversion of lignocellulosic biomass into glucose. Renew Sustain Energy Rev 136:110442. https://doi.org/10.1016/j.rser.2020.110442
31. Ma S et al (2022) Production of biomass-based composite from reed pretreated by ball-milling combined with p-toluenesulfonic acid. Ind Crops Prod 180:114712. https://doi.org/10.1016/j.indcrop.2022.114712
32. Kim SJ et al (2018) Combined ball milling and ethanol organosolv pretreatment to improve the enzymatic digestibility of three types of herbaceous biomass. Energies 11(9):2457. https://doi.org/10.3390/en11092457
33. Zhang M et al (2022) Structural changes and enzymatic saccharification intensification of spent mushroom substrate after ball milling pretreatment. Biomass Convers Bioref 14:1–8. https://doi.org/10.1007/s13399-022-02963-7
34. Zakaria MR et al (2014) Ball milling pretreatment of oil palm biomass for enhancing enzymatic hydrolysis. Appl Biochem Biotechnol 173:1778–1789. https://doi.org/10.1007/s12010-014-0964-5
35. Lin Z et al (2010) Ball milling pretreatment of corn stover for enhancing the efficiency of enzymatic hydrolysis. Appl Biochem Biotechnol 162:1872–1880. https://doi.org/10.1007/s12010-010-8965-5
36. Zhang F et al (2021) Co-production of functional xylo-oligosaccharides and fermentable sugars from corn stover through fast and facile ball mill-assisted alkaline peroxide pretreatment. Biores Technol 337:125327. https://doi.org/10.1016/j.biortech.2021.125327
37. Amorim C et al (2019) From lignocellulosic residues to market: production and commercial potential of xylooligosaccharides. Biotechnol Adv 37(7):107397. https://doi.org/10.1016/j.biotechadv.2019.05.003
38. Gautério GV et al (2022) Xylooligosaccharide production with low xylose release using crude xylanase from Aureobasidium pullulans: effect of the enzymatic hydrolysis parameters. Appl-Biochem Biotechnol 194:1–20
39. Chen L et al (2016) Integrated chemical and multi-scale structural analyses for the processes of acid pretreatment and enzymatic hydrolysis of corn stover. Carbohyd Polym 141(5):1–9. https://doi.org/10.1016/j.carbpol.2015.12.079
40. Nazir MS et al (2023) Eco-friendly extraction and characterization of cellulose from oil palm empty fruit bunches. BioResources 8(2):2161–2172
41. García A et al (2012) Lignin as natural radical scavenger. Effect of the obtaining and purification processes on the antioxidant behaviour of lignin. Biochem Eng J 67(15):173–185. https://doi.org/10.1016/j.bej.2012.06.013
42. Li FH et al (2012) Structure and saccharification of rice straw pretreated with microwave-assisted dilute lye. Ind Eng Chem Res 51(17):6270–6274. https://doi.org/10.1021/ie202547w
43. Ernesto VART et al (2009) Thermal characterization of lignocellulosic residue from different sugarcanes. J Therm Anal Calorim 97(2):653–656. https://doi.org/10.1007/s10973-009-0370-3
44. Ouyang X et al (2015) Improvement of lignin yield and purity from corncob in the presence of steam explosion and liquid hot pressured alcohol. RSC Adv 5(76):61650–61656. https://doi.org/10.1039/C5RA12452B
45. Nakasone K, Ikematsu S, Kobayashi T (2016) Biocompatibility evaluation of cellulose hydrogel film regenerated from sugar cane bagasse waste and its in vivo behavior in mice. Ind Eng Chem Res 55(1):30–37. https://doi.org/10.1021/acs.iecr.5b03926
46. Ju Y et al (2011) Analysis of soluble and insoluble fractions of alkali and subcritical water treated sugarcane bagasse. Carbohyd Polym 83(2):591–599. https://doi.org/10.1016/j.carbpol.2010.08.022
47. Haripriya R et al (2014) Pretreatment of cellulosic waste materials. Eur J Biotech Biosci 2:4–13
48. Xiao X et al (2014) Enhanced enzymatic hydrolysis of bamboo (Dendrocalamus giganteus Munro) culm by hydrothermal pretreatment. Biores Technol 159:41–47. https://doi.org/10.1016/j.biortech.2014.02.096
49. Zhang M et al (2010) Enhanced enzymatic hydrolysis of lignocellulose by optimizing enzyme complexes. Appl Biochem Biotechnol 160:1407–1414. https://doi.org/10.1007/s12010-009-8602-3
50. Le Troedec M et al (2008) Influence of various chemical treatments on the composition and structure of hemp fibres. Compos A Appl Sci Manuf 39(3):514–522. https://doi.org/10.1016/j.compositesa.2007.12.001
51. Hoareau W et al (2004) Sugar cane bagasse and curaua lignins oxidized by chlorine dioxide and reacted with furfuryl alcohol: characterization and stability. Polym Degrad Stab 86(3):567–576. https://doi.org/10.1016/j.polymdegradstab.2004.07.005
52. Xu F et al (2013) Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. Appl Energy 104:801–809. https://doi.org/10.1016/j.apenergy.2012.12.019

53. Faix O (1991) Classification of lignins from different botanical origins by FT-IR spectroscopy. Holzforschung 45(1):21–28. https://doi.org/10.1515/hfsg.1991.45.s1.21
54. Nikonenko NA et al (2000) Investigation of stretching vibrations of glycosidic linkages in disaccharides and polysaccharides with use of IR spectra deconvolution. Biopolymers: Orig Res Biomol 57(4):257–262. https://doi.org/10.1002/1097-0282(2000)57:4%3c257::AID-BIP7%3e3.0.CO;2-3
55. Riley R et al (2014) Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. Proc Natl Acad Sci 111(27):9923–9928. https://doi.org/10.1073/pnas.1400592111
56. Zeng J et al (2014) Effects of lignin modification on wheat straw cell wall deconstruction by Phanerochaete chrysosporium. Biotechnology for Biofuels 7(161). https://doi.org/10.1186/s13068-014-0161-3
57. Seiwert K et al (2021) Development and characterization of whey protein isolate and xylan composite films with and without enzymatic crosslinking. Food Hydrocolloids 120:106847. https://doi.org/10.1016/j.foodhyd.2021.106847
58. Kafle K et al (2015) Effects of delignification on crystalline cellulose in lignocellulose biomass characterized by vibrational sum frequency generation spectroscopy and X-ray diffraction. BioEnergy Research 8:1750–1758. https://doi.org/10.1007/s12155-015-9627-9
59. Wang Z, Zhu X, Deuss PJ (2021) The effect of ball milling on birch, pine, reed, walnut shell enzymatic hydrolysis recalcitrance and the structure of the isolated residual enzyme lignin. Ind Crops Prod 167:113493
60. Sitotaw YW et al (2023) Ball milling as an important pretreatment technique in lignocellulose biorefineries: a review. Biomass Conversion and Biorefinery 13(17):15593–15616