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Chemical input reduction in the arabinoxylan and lignocellulose alkaline extraction and xylooligosaccharides production

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HIGHLIGHTS

- Improvement of alkaline method for obtaining arabinoxylan and lignocellulose.
- Higher temperature, lesser chemicals and shorter time in the efficient extraction.
- Arabinoxylan and lignocellulose extracted in high level from sugarcane bagasse.
- Efficient arabinoxylan hydrolysis for XOS or xylose obtaining by xylanases.
- XOS or xylose produced from arabinoxylan depend on fungal xylanase.

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ABSTRACT

Lignocellulosic material breakdown by hydrolysis is an important step to open new perspectives for bioenergy and special foods production like prebiotic xylooligosaccharides. Improvement of lignocellulose and arabinoxylan alkaline extraction from sugarcane bagasse and enzymatic hydrolysis were performed. Treatments 1 (10% KOH at 70 °C), 3 (5% KOH at 121 °C) and ZD method (24% KOH at 35 °C) showed solid lignocellulose recovery of respectively 75.2%, 74.2% and 73%. A range of 24.8–27% extracted material with high arabinoxylan content (72.1–76.3%) was obtained with these treatments. Treatment 1 and 3 exhibited great KOH reduction in the method reaction, 54.1% and 76.2%, respectively. Likewise, in treatment 3 there was a decrease in ethanol consumption (40.9%) when compared to ZD method. The extracted arabinoxylan showed susceptibility to enzymatic hydrolysis with high solid loading (7%) since *Trichoderma reesei* xylanases were advantageous for xylose production (54.9%), while *Aspergillus fumigatus* xylanases achieved better XOS production (27.1%).

1. Introduction

The lignocellulosic materials (LCM) currently represent the largest biomass source in the world (Carvalho et al., 2013). If conveniently separated into cellulose, xylan and lignin, these materials could be alternatives for the production of food and bioenergy, given the limitations of agricultural areas and the future scarcity of fossil fuels (Kim et al., 2015). In addition, fossil fuels present several disadvantages, such as contributing to climate change, pollution and being a non-renewable resource (Sindhu et al., 2015). Lignin can be burned for bioenergy generation or chemical production, and cellulose and xylan are important substrates for second generation ethanol or opportunities for production of healthier foods (Carvalho et al., 2013).

The main drawback using LCM is due to its recalcitrance characteristic since hemicellulose is found in a complex organization with cellulose and lignin in the cell wall (Kumar et al., 2016; Machado et al., 2016). Besides the recalcitrance, the LCM can be conveniently hydrolyzed with applications in the production of microbial biomass, fermentable sugars, enzymes, bioenergy (biofuels) and many other products (Sharma and Arora, 2013; Weber and Agblevor, 2005; Sun and Cheng, 2002). In addition,

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the lignocellulosic biomass is a renewable alternative for the production of high-value products (Kim et al., 2015; Sindhu et al., 2015; Zhang et al., 2015). However, the LCM hydrolysis is difficult and currently expensive because of the chemical breakdown resistance (Sindhu et al., 2015). Some pretreatments are necessary to break the bonds that link hemicellulose, lignin and cellulose. The pretreatments improve the ability of enzymes to release sugars and other products from LCM (Sindhu et al., 2015).

LCM could be a new source in the nutritional field providing food and nutraceutical ingredients responsible for health benefits, such as prevention of diseases (Hutkins et al., 2016; Bitzios et al., 2011). Nowadays, several studies have been conducted to produce new molecules to modulate the proper functioning of the body (Maslowski and Mackay, 2011). Among these food, supplements are the prebiotics that selectively stimulates the growth of beneficial bacteria, mainly *Bifidobacterium* and *Lactobacillus*, in the intestine of animals (Hutkins et al., 2016; Wasilewski et al., 2015; Al Sheraji et al., 2013).

In the last decades, there was an increasing interest in developing new prebiotic, including the xylooligosaccharides (XOS) (Jain et al., 2015; Rastall and Gibson, 2015; Samanta et al., 2015; Wasilewski et al., 2015). These oligomers are constituted of xylose units linked by β(1-4) bonds, which have beneficial effects on intestinal flora, stimulating a greater number of bifidobacteria in the gut (Chen et al., 2016, 2014; Jain et al., 2015; Mäkeläinen et al., 2010; Gullón et al., 2008). In recent reviews, some health benefits related to the food supplementation with XOS were observed, such as enhancing intestinal functions, calcium absorption and providing positive effects on the immune and cardiovascular system, improving allergenic and anti-inflammatory activities (Brienzo et al., 2016a; Jain et al., 2015; Samanta et al., 2015).

The industrial XOS production can be performed by enzymatic hydrolysis of xylan present in the LCM residues, such as corn cobs, rice husks, barley straw, tobacco stalk, cotton stalk, sunflower stalk, wheat straw and sugarcane bagasse (Samanta et al., 2015). XOS can be obtained directly by acid hydrolysis of LCM, but require additional purification steps (Otieno and Ahring, 2012). Alternatively, XOS can be produced in two stages that consist of the xylan extraction first, which may be an alkaline treatment. In a second stage, an acid or enzymatic hydrolysis of xylan can be performed with a consequent reduction in the purification step requirement (Samanta et al., 2015; Carvalho et al., 2013). The hemicellulose extraction methods and XOS production technology were recently reviewed by Brienzo et al. (2016a).

The breakage of chemical bonds present in LCM is relatively difficult and costly, which limits the solubilization of pure hemicellulose and XOS production with high yield (Brienzo et al., 2016a,b). Therefore, methods that reduce the costs of hemicellulose extraction processes are important for industrial scale. In the present work, the improvement of hemicellulose and lignocellulose extraction aiming at the decrease of chemical consumption and simplification of the process were considered. Moreover, the enzymatic hydrolysis of hemicellulose was analyzed by different important enzymes from fibrolytic microorganisms.

2. Material and method

2.1. Microorganisms

Aspergillus fumigatus M51 and *Trichoderma reesei QM 9414* were obtained from Tropical Culture Collection (CCT), André Tosello Foundation, Campinas, SP, Brazil, with codes CCT 7732 and 2768, respectively. Microorganisms were maintained as a stock culture at 7 °C on Potato Dextrose Agar (PDA).

2.2. Cultivation and enzyme production

A. fumigatus and *T. reesei* strains were cultivated by submerged fermentation (SmF) in Erlenmeyer flasks (250 mL) containing 50 mL medium (m/v): 3.0% napier ground, 0.1% $(NH_4)_2SO_4$, 0.0017% $MgSO_4{\cdot}7H_2O$, 0.1% K_2HPO_4, 0.0028% $ZnSO_4$, 0.1% $NH_4H_2PO_4$, 0.06% KCl, 0.1% yeast extract and 0.1% sucrose at pH 4.5 (Silva et al., 2013). The culture medium was inoculated with 10^6 spores/mL counted by microscopy in a Neubauer chamber. The flasks were incubated at the optimal growth temperature with an orbital shaker (Tecnal mod. 421, Piraciba, Brazil) at 180 rpm for 144 h. The biomass was separated by filtering through gauze and filter paper. The liquid fraction was used as a raw extract of xylanase enzymes.

2.3. Arabinoxylan extraction

The method described by Zilliox and Debeire (1998) (ZD method) was considered reference in this work and was adapted for sugarcane bagasse for arabinoxylan extraction. The sugarcane bagasse (Usina Água Bonita – Tarumã, SP) was washed and grinded, pre-incubated at 60 °C for 16 h in 400 mL of deionized water and afterward drained. After this process, 250 mL of a 24% (m/v) KOH and 1% (m/v) $NaBH_2$ solution was added for reaction with 8% (m/v) sugar cane bagasse during 3 h at 35 °C. The liquid fraction was filtered with gauze until no solids were observed in the liquid phase. The arabinoxylan in the liquid fraction was precipitated by the mixture of 60% (v/v) ethanol, 6.7% (v/v) acetic acid and 33.3% (v/v) arabinoxylan solution. The precipitated material (arabinoxylan) was centrifuged at 4000×*g* for 15 min. This material was washed 4 times with 1 vol ethanol solution (50% ethanol, 50% deionized water and 0.5% EDTA): 1 vol arabinoxylan, before being dried at 60 °C (Zilliox and Debeire, 1998; Akpinar et al., 2009). The scheme in Fig. 1 shows the general sequence of procedures.

Other treatments were applied changing the sugarcane bagasse percentage, with or without pre-incubation at 60 °C for 16 h, the percentage of KOH, temperature, time of reaction, ethanol and acetic acid ratio used for precipitation of arabinoxylan and the number of washes (Table 1). The washes were made until neutral pH has been achieved.

2.4. Enzymatic hydrolysis of arabinoxylan at high solid loading

The enzymatic hydrolysis was conducted applying 120 U/g of endoxylanase, produced by *A. fumigatus* (M51) and *T. reesei* QM 9414, per gram of arabinoxylan extracted (Table 1). The substrate was 20 mL of 7% arabinoxylan in 100 mM acetate buffer at pH 5.5 (Carvalho et al., 2015). The reaction was incubated in a shaker (Tecnal TE 421, Piracicaba, Brazil) at 50 °C with agitation of 130 rpm during 24 h. Samples of 2 mL were collected at 0 h, 12 h and 24 h. Hydrolysis reactions were stopped heating the samples during 10 min in a boiling water bath.

2.5. Analytical methods

2.5.1. Enzymatic activity

Endoxylanase activity was assayed at 50 °C in a reaction with 0.1 mL raw extracts and 0.65 mL of 0.5% xylan Birchwood solution (Sigma-Aldrich) in 250 mM sodium acetate buffer, at pH 5.0 (Bailey et al., 1992). The amount of reducing sugar was quantified by the dinitrosalicylic acid method (Miller, 1959). One unit (U) of xylanase activity was defined as the amount of enzyme that releases 1 μmol of reducing sugar per minute per mL of reaction.

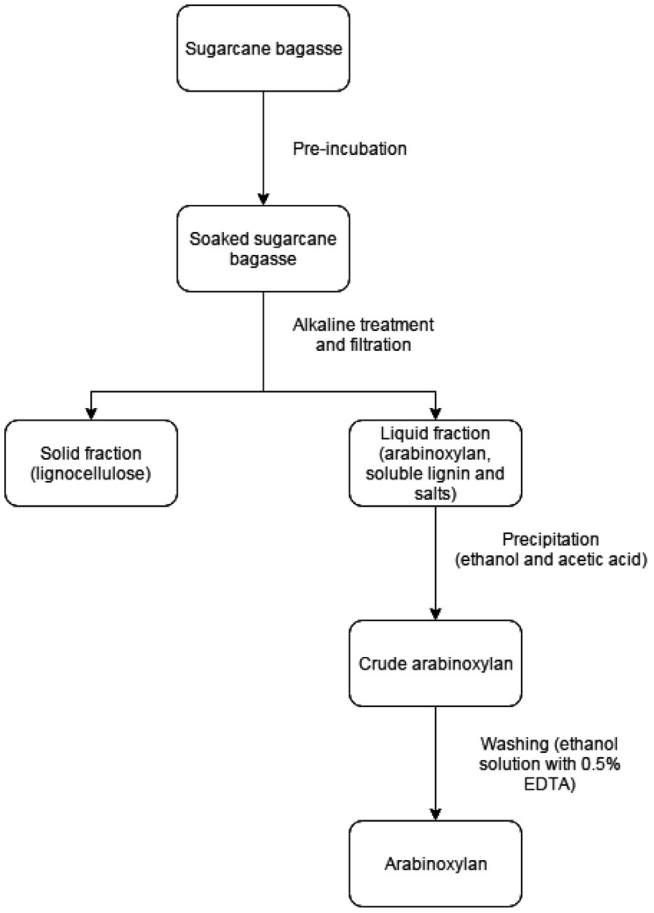

Fig. 1. Schematic representation of arabinoxylan solubilization from sugarcane bagasse for the ZD method and treatments 1, 2 and 3. The experimental condition applied to each treatment is described in the Table 1.

The Yield of Extracted Material (Ym), Yield of Xylan (Yx) and Efficiency in Xylan Extraction (Ex) based on dry mass were calculated by equations:

Eq. (1): $Ym = (Me/Mb) \times 100$, expressed in percentage, where: Me = mass of extracted material (g) and Mb = mass of sugarcane bagasse (g).
Eq. (2): $Yx = (Mx/Mb) \times 100$, expressed in percentage, where: Mx = xylan mass (g) ($Me \times Cx$) and Cx = concentration of xylan in the Me (g × 100 mL).
Eq. (3): $Ex = (Mx/Mxb) \times 100$, expressed in percentage, where: Mxb = mass of xylan quantified in sugarcane bagasse (g) ($Mb \times Cxb$) and Cxb = concentration of xylan in the Mb (g × 100 mL).

2.5.2. Chemical composition of sugarcane bagasse, lignocellulose and arabinoxylan

The moisture content of sugarcane bagasse and the extracted material were obtained by an oven at 105 °C. The percentages of total lignin, arabinoxylan and cellulose (glucan) were obtained according to Sluiter et al. (2008). Total salts were performed at 800 °C for 5 h in a muffle furnace (Marconi MA385, Piracicaba, Brazil), according to biomass ash methodology. The potassium percentage was obtained by the total salts dissolved in HCl with subsequent reading in atomic absorption (Agilent 55B AA).

2.5.3. Xylooligosaccharides determination

The XOS and xylose released in the enzymatic hydrolysis were quantified using High-Performance Liquid Chromatography (HPLC). The analyses were performed with an Agilent 1100 system with a refractive index detector. A Bio-Rad HPX-87C column at 65 °C was eluated with purified water (Milli-Q). The elution time was 20 min with a flow rate of 0.5 mL/min. The standards used were arabinose, xylose (Sigma-Aldrich), xylobiose, xylotriose and xilotetraose (Megazyme International). Total XOS is the sum of oligosaccharides, except xylose. Statistical analyzes were performed to compare the arabinoxylan extraction, production of XOS and xylose using ANOVA by BioEstat 5.0 software.

The yield of XOS and xylose were determined by the following equations:

Eq. (4): XOS (%) = (total XOS (g)/arabinoxylan (g)) × 100
Eq. (5): Xylose (%) = (total xylose (g)/arabinoxylan (g)) × 100

Result and discussion

3.1. Arabinoxylan extraction and chemical characterization

This study focused on the comparison of method conditions to solubilize/extract hemicellulose from sugarcane bagasse. Although there are some reports about the hemicellulose solubilization (Akpinar et al., 2009; Brienzo et al., 2009; Zilliox and Debeire, 1998), the methods condition still prohibitive for industrial application due high chemicals consumption.

The chemical composition of sugarcane bagasse (6% moisture) in this study showed 40.3% cellulose/glucan, 25.1% arabinoxylan (sum of 1.9% arabinan, 0.5% galactan, 21.9% xylan, 0.8% mannan), 25.2% total lignin (1.7% soluble and 23.5% insoluble) and 4.5% ash (Table 2). According to the literature, sugarcane bagasse presents a chemical composition in the range of 24–29% of total arabinoxylan, 19–25% of total lignin, 38–50% of cellulose and 1–7% of ash (Brienzo et al., 2009; Gouveia et al., 2009). The arabinoxylan content in the sugarcane suggests this material is a good source for this macromolecule production. Moreover, the amount of bagasse produced in several countries inspires to studies of the xylan with an industrial application interest.

Table 1
Alkaline treatment for arabinoxylan extraction with the modified experimental conditions.

	Bagasse (%)	Pre-incubation (60 °C/16 h)	KOH (%) (m/m)	Temp. (°C)	Time (h)	Ethanol and acetic acid:hydrolyzed (v/v)	Washes*
ZD method	8	Yes	24	35	3	2:1	5
Treatment 1	8	Yes	10	70	3	2:1	4
Treatment 2	8	Yes	5	70	3	1:1	4
Treatment 3	8	No	5	121	0.5	2:1	1
Treatment 4	12	No	5	121	0.5	2:1	1
Treatment 5	8	No	5	121	0.5	1:1	1

* Xylan washes times with deionized water, ethanol and EDTA; ZD method (Zilliox and Debeire, 1998).

Table 2
Chemical composition of the solid fraction from arabinoxylan extraction.

	Glucan (%)	Lignin (%)	Xylan (%)	Salts total (%)	Others (%)	Solid recovery (%)
Sugarcane bagasse	40.3 ± 0.03	25.2 ± 0.18	25.1 ± 0.66	4.5 ± 0.52	n.s.*	n.s.
ZD method	38.9 ± 0.29ac**	22.8 ± 0.10ad	7.0 ± 0.27ad	3.7 ± 0.05a	0.6 ± 0.34a	73 ± 0.46a
Treatment 1	39.9 ± 0.21b	22.4 ± 0.08b	6.8 ± 0.18a	3.7 ± 0.04a	2.4 ± 0.25a	75.2 ± 0.60be
Treatment 2	39.9 ± 0.30ab	23.2 ± 0.12c	8.8 ± 0.29b	3.4 ± 0.16b	8.7 ± 0.41b	84 ± 0.75c
Treatment 3	39.3 ± 0.54ab	22.6 ± 0.12ab	6.0 ± 0.37c	3.9 ± 0.02c	2.4 ± 0.76a	74.2 ± 0.92ab
Treatment 4	39.2 ± 0.2abc	22.7 ± 0.13a	7.7 ± 0.19d	3.9 ± 0.01c	8.6 ± 0.14b	82 ± 0.04d
Treatment 5	38.3 ± 0.22c	23.0 ± 0.04 cd	8.6 ± 0.43b	3.7 ± 0.01a	3.0 ± 0.19a	76.5 ± 0.08e

* Data not shown.
** Values followed by the same letter were not significantly different in ANOVA followed by Tukey test.

Table 3
Yield of Extracted Material (Ym), yield of arabinoxylan (Yx) and Efficiency in Xylan Extraction (Ex) from sugarcane bagasse.

	Yield of extracted material (Ym %)	Yield of xylan (Yx %)	Efficiency in xylan extraction (Ex %)
ZD method	27.0 ± 1.9a*	19.6 ± 0.3a	78.0 ± 0.2a
Treatment 1	24.8 ± 0.9a	18.0 ± 0.2a	72.0 ± 0.1a
Treatment 2	16.0 ± 1.2b	10.3 ± 0.5b	41.3 ± 0.3b
Treatment 3	25.8 ± 1.6a	18.3 ± 0.2a	72.9 ± 0.1a
Treatment 4	18.0 ± 0.2b	12.6 ± 0.1c	50.1 ± 0.1c
Treatment 5	23.5 ± 0.1a	15.4 ± 0.3d	61.6 ± 0.2d

* Values followed by the same letter were not significantly different in ANOVA followed by Tukey test.

The yield of extracted material (Ym) from sugarcane bagasse was statistically similar to the treatments 1, 3 and ZD method, respectively 24.8%, 25.8% and 27% (Table 3). The treatment 2, 4 and 5 showed lower Ym compared to treatment 1, 3 and ZD. Similar trend was observed for a yield of xylan (Yx) and Efficiency in xylan extraction (Ex). As industrial application desires, the solid loading or sugarcane percentage was increased. The increase in the sugarcane bagasse solid loading to 12% in the treatment 4 did not result in an increase in the percentage of extracted material, yield of xylan or efficiency in xylan extraction, 18%, 12.6% and 50.1%, respectively (Table 3). These results were probably due to an increase in the acetic acid available to react with the KOH since the consumption of this acid in the KOH reaction did not increased. Other limitation could be the high viscosity (data not shown) which can difficult the KOH catalyst action (Jorgensen et al., 2006).

The treatment 2 showed the lower results for yield of extracted material (16%), Yx (10.3%) and Ex (41.3%) (Table 3). The treatment 5 showed a good Ym with 23.5%, close to the values of the treatments 1, 3 and ZD. However, the arabinoxylan content in the material extracted was lower (61.6%) compared to the treatments 1, 3, 4 and ZD method (Table 3).

The treatment 1, which used 54.1% lower KOH than ZD method (Table 5), showed similar results of arabinoxylan concentration in the extracted material (purity), with 72.8% and 72.1%, respectively (Table 4). However, treatment 3 showed a slightly but statistically higher arabinoxylan concentration (76.3%) (Table 4). Except for treatments 1 and 4, all the treatments showed statistically significant differences in arabinoxylan concentration/purity (ANOVA and Tukey test $p < 0.05$, 95% confidence level) compared to ZD method. These differences in the treatments with higher arabinoxylan content (1, 3 and ZD method) compared to treatments 2 and 5 could be related to the proportion of ethanol and acetic acid used during the precipitation process.

The proportion of only 40% (v/v) of ethanol and 10% (v/v) of acetic acid to 50% (v/v) of arabinoxylan solution were used in the treatments 2 and 5. On the other hand, 60% (v/v) ethanol and 6.67% (v/v) acetic acid to 33.3% (v/v) arabinoxylan solution were used in the treatments 1, 3 and ZD method. The data indicate the need for a higher proportion of ethanol and lower concentration of acetic acid to arabinoxylan separation. Another important aspect was a lower salt residue after precipitation, verified in the treatments in which more solvents were used (treatments 1, 3, 4 and ZD method) and consequently, a simpler wash step decreased the ethanol consumption.

Treatments 1, 3 and ZD method showed greater Yx values (18%, 18.3% and 19.6%, respectively) and Ex values (72%, 72.9% and 78%, respectively) (Table 3). If the arabinoxylan content in the treatment 3 is considered (76.3%), this result was higher than the extraction with 12% KOH combined with steam explosion of the sugarcane bagasse, since only 53% of xylan content was extracted (Jayapal et al., 2013). The xylan extraction (71.9%) from sugarcane bagasse using ZD method as reported by Carvalho et al. (2015) was also lower than treatment 3. However, a combination of steam explosion and a higher 12% NaOH could be responsible for higher xylan content (85%) extracted (Jayapal et al., 2013).

3.2. Solid recovery

Although a higher amount of arabinoxylan can be extracted with a alkaline method, it still prohibitive for industrial scale. On the other hand, method combination could be applied in the process where more than a macromolecule is the target. From this perspective, this topic evaluated the solid recovery from the arabinoxylan extraction.

A range of 73 to 84% of the fraction containing water insoluble solids or lignocellulose was obtained after hemicellulose extraction. These materials showed composition of 38.3–39.9% of glucan

Table 4
Chemical composition of extracted material using ZD method and treatments 1–5.

	Arabinoxylan (%)	Glucan (%)	Lignin Total (%)	Salts total (%)	Potassium (%)**
ZD method	72.1 ± 0.6ad*	3.4 ± 0.2ac	9.4 ± 0.7ad	16.13 ± 0.6a	2.27 ± 0.3a
Treatment 1	72.8 ± 0.3a	1.10 ± 0.5b	11.2 ± 0.2b	16.54 ± 0.5a	2.18 ± 0.1ab
Treatment 2	64.8 ± 0.9b	1.08 ± 0.5ab	7.8 ± 0.2 cd	22.09 ± 0.3b	2.49 ± 0.2bd
Treatment 3	76.3 ± 0.4c	2.40 ± 1.3ab	10.3 ± 0.3ab	13.06 ± 0.1c	1.11 ± 0.5c
Treatment 4	69.5 ± 0.2d	2.72 ± 0.6ab	10.0 ± 0.3abe	12.07 ± 0.3c	1.00 ± 0.1c
Treatment 5	65.9 ± 0.6b	4.88 ± 0.5c	8.7 ± 0.1de	16.82 ± 0.2a	1.86 ± 0.2d

* Values followed by the same letter were not significantly different in ANOVA followed by Tukey test.
** Based on total salts.

Table 5
Comparison of inputs used in different alkaline treatments for arabinoxylan extraction.

	Ethanol/Arabinoxylan (mL/g)				Total KOH/xylan (g/g)	KOH reduction (%)	Total time (h)*
	Precipitation	Washes	Total	Reduction/increase (%)			
ZD method	52.9	73.5	126.4	0	11.76	0	20.25
Treatment 1	58.7	65.2	123.9	−1.98	5.40	54.1	20
Treatment 2	40.0	100	140.0	+10.8	4.16	64.6	20
Treatment 3	60.0	14.7	74.7	−40.9	2.80	76.2	0.75
Treatment 4	52.9	14.7	67.6	−46.5	2.45	79.2	0.75
Treatment 5	27.3	17	44.3	−65.0	2.84	75.9	0.75

* Considering pre-incubation (0–16 h), alkaline extraction (30 min–3 h) and washes (15 min/wash), except precipitation (15 min) and drying (24 h), because both are the same in all experiments.

Table 6
XOS and xylose production from enzymatic hydrolysis of arabinoxylan from sugarcane bagasse.

Microorganisms	KOH (%)	Extraction temperature (°C)	Arabinoxylan hydrolysis (%)*	Xylose (%)	XOS (%)
T. reesei					
ZD method 0 h	24	35	0	0	0
ZD method 12 h	24	35	21.90 ± 0.08	6.79 ± 0.09	15.05 ± 0.07
ZD method 24 h	24	35	40.30 ± 0.75	28.73 ± 1.94	11.57 ± 0.15
Treatment 1 0 h	10	70	0	0	0
Treatment 1 12 h	10	70	21.64 ± 0.14	7.29 ± 0.10	14.36 ± 0.15
Treatment 1 24 h	10	70	55.50 ± 0.14	42.51 ± 0.10	12.96 ± 0.16
Treatment 3 0 h	5	121	0	0	0
Treatment 3 12 h	5	121	26.91 ± 0.08	9.09 ± 0.08	17.83 ± 0.08
Treatment 3 24 h	5	121	70.61 ± 0.16	54.91 ± 0.29	15.70 ± 0.10
A. fumigatus					
ZD method 0 h	24	35	0	0	0
ZD method 12 h	24	35	17.76 ± 0.16	0.03 ± 0.19	17.73 ± 0.08
ZD method 24 h	24	35	54.91 ± 0.21	28.93 ± 0.44	25.99 ± 0.12
Treatment 1 0 h	10	70	0	0	0
Treatment 1 12 h	10	70	53.34 ± 0.07	28.33 ± 0.17	25.87 ± 0.02
Treatment 1 24 h	10	70	46.53 ± 0.51	19.60 ± 0.78	27.10 ± 0.12
Treatment 3 0 h	5	121	0	0	0
Treatment 3 12 h	5	121	44.84 ± 0.10	26.33 ± 0.19	18.51 ± 0.06
Treatment 3 24 h	5	121	38.21 ± 1.24	19.23 ± 0.44	18.99 ± 0.14

* Based on dry mass used in the hydrolysis; 7% of arabinoxylan, 120 U of xylanases per gram of substrate, pH 5.5, 130 rpm, at 50 °C.

and 22.4–23.2% of lignin, with arabinoxylan content lower than 9% (Table 2). Considering the glucan and lignin content in the raw sugarcane bagasse, respectively 40.3% and 25.2%, there was a great recovery of both components. In fact, the content of lignin and residual arabinoxylan was similar for all the treatments applied in this study. The results showed around 22% of lignin and 8% of arabinoxylan, with some statistical differences for materials from ZD method, treatments 1 and 3. For lignin, treatment 1 was statistically different from ZD and treatment 3, while these two were similar. However, for arabinoxylan, ZD and treatment 1 were similar, while treatment 3 displayed a statistically significant difference (Table 2). In order to evaluate if cellulose can be easily released by enzymatic hydrolysis, further tests with cellulases are necessary.

Moreover, the literature reports indicate the success of using KOH, even compared to NaOH, in the pretreatment of rice straw and corn over enzymatic hydrolysis of the cellulose (Ong et al., 2010; Kaar and Holtzapple, 2000). 0.5% KOH at 21 °C during 12 h was enough for provide good digestibility of the cellulose (Sharma et al., 2013). Comparing these conditions with the present work, the time was longer but the concentration of the alkali was lower. According to Wu et al. (2011) sugarcane bagasse treated with NaOH during a 30 min to 2 h period at room temperature showed low loss of glucan, since 95% of glucose was recovered from the original in the lignocellulosic biomass. Xylan and lignin were greatly solubilized, with a maximum of 75% of xylan extraction with 2.5 M NaOH, improving the enzymatic hydrolysis of the cellulose. A study using corncob pretreated with a combination of 2% H_2SO_4 and 2% NaOH in high temperatures (80–121 °C) for 45 min resulted in a recovery of 91.1% of glucan and this combination was effective in removing lignin and hemicellulose, consequently making the enzymatic process easier (Zhang et al., 2010).

3.3. Chemicals input and reaction time reduction

The treatment conditions can influence the arabinoxylan extraction yield and quality, e.g. the severity of the process. The higher severity could result in higher macromolecule extraction and cellulose digestibility. However, moderate conditions could result in comparable extraction yield and quality. Decreasing the chemical input, water use, and reaction time can make the industrial scale up viable. In order to determine cost of these extraction, considering input-output chemicals/components, a properly economic evaluation is necessary.

The treatments evaluated showed an overall reduction in the chemicals required and time consumed. The treatments 3, 4 and 5 showed over 75% reduction of KOH per gram of extracted arabinoxylan and an ethanol reduction over 40% in relation to ZD method (Table 5). For these treatments, the reaction time was a 27 times lower than ZD method. In addition, the removal of the pre-incubation step and reduction of 4 washes simplified the process. In the treatments 1 and 2, the reaction time was similar to ZD method, saving only 15 min provided by the removal of 1 wash. Although the increase of 35 °C (ZD method) to 70 °C (treatments 1 and 2) and 121 °C (treatments 3, 4 and 5) resulted in an increase of energy used, all treatments showed reduction of the chemical inputs in relation to ZD method. The treatment 2 can be exclude since there was a 10.8% increase in ethanol consumption (Table 5).

In conclusion treatments 1 and 3 showed better results for arabinoxylan extraction based on: a) higher arabinoxylan percentage (Table 4); b) similar salt content in arabinoxylan extracted from these treatments when compared with ZD method (Table 4); c) decrease in the chemicals used since treatment 1 reduced KOH by 54.1% and ethanol consumption by 1.98%, and treatment 3 decreased KOH by 76.2% and ethanol consumption by 40.9% (Table 5); d) decrease of time from 20.25 h (ZD method) to 0.75 h (treatment 3) (Table 5).

3.4. Enzymatic hydrolysis of the arabinoxylan

Probably one of the most interesting uses of arabinoxylan is via enzymatic hydrolysis process. To evaluate the susceptibility of the extracted arabinoxylan as influenced by the different treatments, xylanases from fungal raw extracts were applied. Both the xylose and xylooligosaccharides, products for bioenergy and food industry respectively, were evaluated.

The enzymatic hydrolysis was carried out with fungal enzymes of *T. reesei* and *A. fumigatus* using the arabinoxylan extracted from ZD method and the treatments 1 and 3 as substrate. A high xylose production and low release of xylobiose (X2), xylotriose (X3) and a trace of XOS with a higher degree of polymerization (DP) were obtained from *T. reesei* enzymes hydrolysis.

The highest percentage of arabinoxylan hydrolysis was observed in 24 h of reaction with *T. reesei* enzymes. The effect of KOH decrease with the increase of temperature in the arabinoxylan extraction improved the enzymatic hydrolysis and conversion into xylose and XOS, since ZD method, treatment 1 and 3 showed respectively 40.3%, 55.5% and 70.6% (Table 6). The highest yields of xylose were shown in 24 h of reaction, with 28.7% in the ZD method, 42.5% in the treatment 1 and 54.9% in the treatment 3.

The XOS production was higher at 12 h of reaction at a similar level to the ZD method and treatment 1, with a yield of 15% and 14.4%, respectively. These enzymatic hydrolyses were with 7% arabinoxylan, and the main products were X2 and X3. In treatment 3, the XOS production was slightly higher since 17.8% and 15.7% was obtained in 12 and 24 h, respectively (Table 6). A greater XOS production (20%) in a short reaction time (6 h) was obtained using *T. reesei* enzymes and 3.8% xylan (Brienzo, 2010). The author concluded that increasing the reaction time from 6 to 24 h favored the release of xylose. This fact could be explained by the higher reaction time stimulating the action of β-xylosidase to breakdown the XOS, releasing xylose. In addition, *T. reesei* is a producer of β-xylosidase (Poutanen and Puis, 1988; Mata et al., 1992) and xylanase enzymes. According to the literature, studies with other microorganisms reported that high concentrations of xylose inhibit the xylanase activity, which converts xylan into XOS (Aro et al., 2005; Vázquez et al., 2000).

The enzymatic hydrolysis performed with *A. fumigatus* M51 enzymes released a higher amount of X2 and X3. In this reaction XOS with higher DP was not detected. The xylose released was in lower amounts, except for the arabinoxylan extracted with the ZD method (Table 6). The arabinoxylan hydrolysis extracted by the treatments 1 and 3 showed the higher percentage of hydrolysis in 12 h, with 53.3% and 44.8%, respectively (Table 6). The ZD method showed a similar percentage of arabinoxylan hydrolysis (54.9%) in 24 h of reaction to the treatment 1 in 12 h. Unlike the previous hydrolysis, the increase in temperature and decrease of KOH consumption in arabinoxylan extractions decreased the percentage of enzymatic hydrolysis. The highest yield of xylose was verified with arabinoxylan from the treatment 1 (28.3%) and 3 (26.3%) in 12 h of reaction (Table 6). The xylose release profile was different from the ZD method, which showed low release after 12 h but a peak of xylose (28.9%) in 24 h.

For all arabinoxylans extracted, XOS production using *A. fumigatus* enzymes was higher than *T. reesei* enzymes. The releases of XOS were 26.0%, 27.1% and 19%, respectively using arabinoxylan from ZD method, treatment 1 and 3 in 24 h (Table 6). These results were higher than the yield of only 8.6% XOS using 2% xylan extracted by alkaline pretreatment of sugarcane bagasse and enzymes of *Trichoderma viridae* in 8 h of reaction (Jayapal et al., 2013). In another study, using tobacco stalk as a substrate for xylan extraction through alkaline method, only 11.4% of XOS was obtained with enzymes of *Aspergillus niger* after 24 h of reaction at 40 °C (Akpinar et al., 2009). However, a longer reaction time (48–96 h) and lower substrate concentration (2% xylan extracted from sugarcane bagasse) were necessary to obtain 37.1–37.6% of XOS yield at 50 °C using enzymes of *A. fumigatus* (Carvalho et al., 2015) or *Thermoascus aurantiascus* (Brienzo et al., 2010).

The enzymatic hydrolysis with *T. reesei* enzymes showed were shown to be advantageous for xylose production, particularly when using arabinoxylan treated with 5% KOH at 121 °C for 30 min. On the other hand, experiments with enzymes of *A. fumigatus* were shown to be more efficient for the XOS production, especially xylobiose and xylotriose.

Although the XOS production with enzymes of *A. fumigatus* did not present a statistically significant difference (ANOVA $p = 0.28$) in the enzymatic reactions with arabinoxylan extracted from different treatments, the treatment with 10% KOH exhibited a more interesting result for the efficient XOS production with lower release of xylose and the reduction in the amount of KOH, resulting in purer product and significant savings with this chemical (Table 5).

4. Conclusions

Alkaline method for arabinoxylan extraction from LCM depends on temperature to reduce inputs, such as KOH and ethanol. Water insoluble solids obtained after the arabinoxylan extraction showed higher mass recovery with great glucan and lignin content. Hydrolysis with *A. fumigatus* enzymes presented a higher XOS yield, while enzymatic hydrolysis with *T. reesei* enzymes was advantageous for xylose production. Altogether, treatment with moderate conditions provided high arabinoxylan extraction with good susceptibility to enzyme action for production of both xylose and XOS. In addition, the recovered solid is rich in cellulose and lignin, with possible process integration for use of these macromolecules.

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References

Akpinar, O., Erdogan, K., Bostanci, S., 2009. Enzymatic production of xylooligosaccharide from selected agricultural wastes. Food Bioprod. Process. 87, 145–151.
Al Sheraji, S.H., Ismail, A., Manap, M.Y., Mustafa, S., Yusof, R.M., Hassan, F.A., 2013. Prebiotics as functional foods: a review. J. Funct. Foods 5, 1542–1553.
Aro, N., Pakula, T., Pentilla, M., 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. Fems Microbiol. Rev. 29, 719–739.
Bailey, M.J., Biely, P., Poutanen, K., 1992. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol. 23, 257–270.
Bitzios, M., Fraser, I., Haddock-Fraser, J., 2011. Functional ingredients and food choice: results from a dual-mode study employing means-end-chain analysis and a choice experiment. Food Policy 36, 715–725.
Brienzo, M., 2010. Hemicellulose Extraction from Sugarcane Bagasse for Xylooligosaccharide Production (Ph.D. thesis). Engineering School of Lorena, University of São Paulo (USP), Brazil.
Brienzo, M., Siqueira, A.F., Milagres, A.M.F., 2009. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. Biochem. Eng. J. 46, 199–204.

Brienzo, M., Carvalho, W., Milagres, A.M.F., 2010. Xylooligosaccharides production from alkali-pretreated sugarcane bagasse using xylanases from *Thermoascus aurantiacus*. Appl. Biochem. Biotechnol. 162, 1195–1205.

Brienzo, M., Carvalho, A.F.A., Figueiredo, F.C., Oliva Neto, P., 2016a. Sugarcane bagasse hemicellulose properties, extraction technologies and xylooligosaccharides production. In: Riley, G.L. (Ed.), Food Waste: Practices, Management and Challenges. Nova Science Publishers, New York, pp. 155–188.

Brienzo, M., Abud, Y., Ferreira, S., Corrales, R.C.N.R., Ferreira-Leitão, V.S., De Souza, W., Sant'Anna, C., 2016b. Characterization of anatomy, lignin distribution, and response to pretreatments of sugarcane culm node and internode. Ind. Crops Prod. 84, 305–313.

Carvalho, A.F.A., Oliva Neto, P., Silva, D.F., Pastore, G.M., 2013. Xylo-oligosaccharides from lignocellulosic materials: chemical structure, health benefits and production by chemical and enzymatic hydrolysis. Food Res. Int. 51, 75–85.

Carvalho, A.F.A., Oliva Neto, P., Almeida, P.Z., Silva, J.B., Escaramboni, B., Pastore, G. M., 2015. Screening of xylanolytic *Aspergillus fumigatus* for prebiotic xylooligosaccharide production using bagasse. Food Technol. Biotechnol. 53, 428–435.

Chen, M.H., Bowman, M.J., Dien, B.S., Rausch, K.D., Tumbleson, M.E., Singh, V., 2014. Autohydrolysis of *Miscanthus* × *giganteus* for the production of xylooligosaccharides (XOS): kinetics, characterization and recovery. Bioresour. Technol. 155, 359–365.

Chen, M.H., Bowman, M.J., Cotta, M.A., Dien, B.S., Iten, L.B., Whitehead, T.R., Rausch, K.D., Tumbleson, M.E., Singh, V., 2016. *Miscanthus* × *giganteus* xylooligosaccharides: purification and fermentation. Carbohydr. Polym. 140, 96–103.

Gouveia, E.R., Do Nascimento, R.T., Souto-Maior, A.M., 2009. Validation of sugarcane bagasse chemical characterization method. Quim. Nova 32, 1500–1503.

Gullón, P., Moura, P., Esteves, M.P., Girio, F.M., Domínguez, H., Parajó, J.C., 2008. Assessment on the fermentability of xylooligosaccharides from *Rice husks* by probiotic bacteria. J. Agric. Food Chem. 56, 7482–7487.

Hutkins, R.W., Krumbeck, J.A., Bindels, L.B., Cani, P.D., Fahey Jr., G., Goh, Y.J., Hamaker, B., Martens, E.C., Mills, D.A., Rastal, R.A., Vaughan, E., Sanders, M.E., 2016. Prebiotics: why definitions matter. Curr. Opin. Biotechnol. 37, 1–7.

Jain, I., Kumar, V., Satyanarayana, T., 2015. Xylooligosaccharides: an economical prebiotic from agroresidues and their health benefits. Indian J. Exp. Biol. 53, 131–142.

Jayapal, N., Samanta, A.K., Kolte, A.P., Senani, S., Sridhar, M., Suresh, K.P., Sampath, K. T., 2013. Value addition to sugarcane bagasse: xylan extraction and its process optimization for xylooligosaccharides production. Ind. Crops Prod. 42, 14–24.

Jorgensen, H., Vibe-Pedersen, J., Larsen, J., Felby, C., 2006. Liquefaction of lignocellulose at high-solids concentrations. Biotechnol. Bioeng. 96, 862–870.

Kaar, W.E., Holtzapple, M.T., 2000. Using lime pretreatment to facilitate the enzymatic hydrolysis of corn stover. Biomass Bioenergy 18, 189–199.

Kim, J.S., Lee, Y.Y., Kim, T.H., 2015. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour. Technol. 199, 42–48.

Kumar, R., Tabatabaei, M., Karimi, K., Horváth, I.S., 2016. Recent updates on lignocellulosic biomass derived ethanol – a review. Biofuel Res. J. 9, 347–356.

Machado, G., Leon, S., Santos, F., Lourega, R., Dullius, J., Mollmann, M.E., Eichler, P., 2016. Literature review on furfural production from lignocellulosic biomass. Nat. Resour. 7, 115–129.

Mäkeläinen, H., Forssten, S., Saarinen, M., Stowell, J., Rautonen, N., Ouwehand, A.C., 2010. Xylo-oligosaccharides enhance the growth of bifidobacteria and *Bifidobacterium lactis* in a simulated colon model. Benef. Microbes 1, 81–91.

Maslowski, K.M., Mackay, C.R., 2011. Diet, gut microbiota and immune responses. Nat. Immunol. 12, 5–9.

Mata, I., Estrada, P., Macarrón, R., Dominguez, J.M., Castillon, P., Acebal, C., 1992. Chemical mechanism of β-xylosidase from *Trichoderma reesei* QM 9414: pH-dependence of kinetic parameters. Biochem. J. 283, 679–682.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Ong, L.G.A., Chuah, C., Chew, A.L., 2010. Comparison of sodium hydroxide and potassium hydroxide followed by heat treatment on rice straw for cellulase production under solid state fermentation. J. Appl. Sci. 10, 2608–2612.

Otieno, D.O., Ahring, B.K., 2012. A thermochemical pretreatment process to produce xylooligosaccharides (XOS), arabinooligosaccharides (AOS) and mannooligosaccharides (MOS) from lignocellulosic biomasses. Bioresour. Technol. 112, 285–292.

Poutanen, K., Puis, J., 1988. Characteristics of *Trichoderma reesei* p-xylosidase and its use in the hydrolysis of solubilized xylans. Appl. Microbiol. Biotechnol. 28, 425–432.

Rastall, R.A., Gibson, G.R., 2015. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Curr. Opin. Biotechnol. 32, 42–46.

Samanta, A.K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A.P., Senani, S., Sridhar, M., 2015. Xylooligosaccharides as prebiotics from agricultural by-products: production and applications. Bioact. Carbohydr. Dietary Fibre 5, 62–71.

Sharma, R.K., Arora, D.S., 2013. Fungal degradation of lignocellulosic residues: an aspect of improved nutritive quality. Crit. Rev. Microbiol. 41, 52–60.

Sharma, R., Palled, V., Sharma-Shivappa, R., Osborne, J., 2013. Potential of potassium hydroxide pretreatment of switchgrass for fermentable sugar production. Appl. Biochem. Biotechnol. 169, 761–772.

Silva, D.F., Camargo, R., Carvalho, A.F.A., Oliva Neto, P., 2013. Cellulolytic enzyme production by the fungi *Trichoderma reesei* QM9414 and *Trichoderma reesei* CCT2768 using citrus pulp. In: Poster presented at the 6th Symposium of Applied Microbiology. Rio Claro, SP, Brazil.

Sindhu, R., Binod, P., Pandey, A., 2015. Biological pretreatment of lignocellulosic biomass – an overview. Bioresour. Technol. 199, 76–82.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008. Determination of Structural Carbohydrates and Lignin in Biomass. LAP-002 NREL Analytical Procedure. National Renewable Energy Laboratory, Golden, CO.

Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol. 83, 1–11.

Vázquez, M.J., Alonso, J.L., Domínguez, H., Parajó, J.C., 2000. Xylooligosaccharides: manufacture and applications. Trends Food Sci. Technol. 11, 387–393.

Wasilewski, A., Zielínska, M., Storr, M., Fichna, J., 2015. Beneficial effects of probiotics, prebiotics, synbiotics, and psychobiotics in inflammatory bowel disease. Inflamm. Bowel Dis. 21, 1674–1682.

Weber, J., Agblevor, F.A., 2005. Microbubble fermentation of *Trichoderma reesei* for cellulase production. Process Biochem. 40, 669–676.

Wu, L., Arakane, M., Ike, M., Wada, M., Takai, T., Gau, M., Tokuyasu, K., 2011. Low temperature alkali pretreatment for improving enzymatic digestibility of sweet sorghum bagasse for ethanol production. Bioresour. Technol. 102, 4793–4799.

Zhang, M., Wanga, F., Su, R., Qi, W., He, W., 2010. Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. Bioresour. Technol. 101, 4959–4964.

Zhang, K., Pei, Z., Wang, D., 2015. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review. Bioresour. Technol. 199, 21–33.

Zilliox, C., Debeire, P., 1998. Hydrolysis of wheat straw by a thermostable endoxylanase: adsorption and kinetic studies. Enzyme Microb. Technol. 22, 58–63.