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S.I.: DIVERSIFICATION OF SUGAR CROPS FOR VALUE ADDITION

Xylitol Production from Sugarcane Bagasse Through Ultrasound-Assisted Alkaline Pretreatment and Enzymatic Hydrolysis Followed by Fermentation

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Abstract This study focused on the optimization of xylitol production from sugarcane bagasse by using response surface methodology. Xylitol was produced through a series of processes, firstly, optimization of ultrasound-assisted mild alkaline pretreatment for the xylan extraction from sugarcane bagasse followed by enzymatic hydrolysis of xylan to xylose by enzyme β-1,4-xylanase and finally microbial fermentation of xylose to xylitol using yeast (*Candida guilliermondii*), bacteria (*Corynebacterium glutamicum*) and their mixed culture for different time periods (0–96 h). Maximum xylan recovery of 12.059% (w/w) was observed at pretreatment; 0.73 M NaOH, 1:38.55 solid-to-liquid ratio and 34.77 min ultrasonication. The enzyme concentration of 400 U/g xylan at 48 h of incubation showed the highest xylose production (81.51 mg/g bagasse). Yeast (*C. guilliermondii*) resulted in the highest xylitol yield (Yp/s = 0.43 g/g) after 72 h. This bioprocess route can contribute as a suitable alternative for chemical methods of xylitol production.

Keywords Xylitol · Xylan · Response surface methodology · Enzymatic hydrolysis · Sugarcane bagasse

Introduction

Sugarcane (*Saccharum species hybrid*) is one of the major tropical crops with the world's annual production of around 1.81 billion tons in the year 2015, which is presumed to reach above 2.21 billion tons by 2024, based on which sugarcane bagasse production is supposed to reach 0.6 billion tons (Martinez-hernandez et al. 2018; Chandel et al. 2012). Sugarcane bagasse is the main byproduct of sugar industry which generates around 280 kg of bagasse per ton of sugarcane processing (Cardona et al. 2010). This implies the necessity of waste valorization of sugarcane bagasse (Cardona et al. 2010) as lack of proper utilization and irrational disposal of biological waste pose a great environmental threat (Albuquerque et al. 2014). Sugarcane bagasse is a lignocellulosic material with high hemicellulose content (Vallejos et al. 2016). The hemicellulose-rich agroindustrial wastes are good source of D-xylose (Rao et al. 2006) which can be hydrolyzed into xylitol by microbial fermentation and enzymatic conversion (Prakash et al. 2011).

Xylitol ($C_5H_{12}O_5$) refers to a polyalcohol having a hydroxyl group connected to each carbon atom in their chain. The relative sweetness of xylitol is equivalent to that of sucrose, but it exerts nearly one third low calorie content (Dasgupta et al. 2017; Nabors 2001). Commercially, xylitol has extensive application in various sectors of pharmaceuticals, nutraceuticals, food and beverage industries due to its distinct pharmacological importance like prevention of dental cavities and ear infection in small children (O'Donnell and Kearsley 2012); low glycemic index (Elamin et al. 2012); higher cooling power (Mussatto and Roberto 2002); independent of insulin metabolic pathway (Chen et al. 2010); and anticariogenic property (Dasgupta et al. 2017).

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The conventional acid hydrolysis method to produce xylitol involves high concentration of acid at high temperature leading to the formation of various toxic compounds hindering the fermentation and purification process (Mohamad et al. 2015). Similarly, the efficiency of solo enzymatic hydrolysis to yield xylose is reduced due to the hindrance of lignin for efficient enzyme penetration into the lignocellulosic biomass (Rafiqul and Sakinah 2013). Hemicellulose is strongly bound to cellulose via physical interaction and hydrogen bonding and to lignin via covalent bonding that causes hindrance in the complete recovery of xylan from lignocellulosic biomass (Jayapal et al. 2013). Hence, alkali treatment for xylan recovery can be combined with ultrasound treatment (Velmurugan and Muthukumar 2012). Mild pretreatment leading to detaching lignin, lowering cellulose crystallinity and increasing porosity of the matrix before enzymatic hydrolysis has been reported to increases extraction efficiency (Rafiqul and Sakinah, 2013). Biotechnological methods such as fermentation and enzymatic hydrolysis for D-xylose to xylitol conversion are frequently studied (Albuquerque et al. 2014). The utilization of different strains of yeast, bacteria and fungi have been reported for the fermentation of D-xylose to xylitol. *Candida guilliermondii* and *C. tropicalis* were reported as efficient xylitol producers (Rafiqul and Sakinah 2013). However, bacteria and fungi are found to have lower xylitol production compared to yeast (Mohamad et al. 2015).

The aim of this study was to optimize the ultrasound-assisted alkaline pretreatment and enzymatic hydrolysis of sugarcane bagasse for xylose recovery and to investigate the effect of different starter cultures of yeast (*Candida guilliermondii*), bacteria (*Corynebacterium glutamicum*) and their mixed culture on the fermentation of xylose-rich sugarcane bagasse hydrolysates to produce xylitol.

Materials and Methods

Materials

Sugarcane bagasse was provided by Mitr Phol Sugar Corporation Limited, Thailand. *Candida guilliermondii* (TISTR 5068) and *Corynebacterium glutamicum* (TISTR 461) were acquired from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Enzyme, β-1,4-xylanase refined from *Trichoderma reesei* was obtained from CTi & Science Co. Ltd., Thailand. All other chemicals used were of analytical grade.

Compositional Analysis of Sugarcane Bagasse Powder

Sugarcane bagasse was dried in an oven at 60 ± 5 °C (Memmert GmbH + Co. KG, D-91126 Schwabach, Germany) and ground with hammer mill (Polymix, PX-MFC 90 D, Kinematica AG, Switzerland) into small fine particles of mesh size 0.5 mm. The compositional analysis (cellulose, hemicellulose, lignin, ash and extractives) of untreated raw sugarcane bagasse was conducted by using wet chemical method as described by Carrier et al. (2011) with slight modifications. Acetone treatment was used for the estimation of extractives, sulfuric acid treatment (72%) for lignin estimation and NaOH (0.5 M) treatment for hemicellulose estimation. Ash content was determined by incineration of sample in a muffle furnace at 575 °C and the final cellulose content was calculated by difference, assuming that the entire biomass is comprised of cellulose, hemicellulose, lignin, extractives and ash. The proximate analysis of sugarcane bagasse was performed by the proximate analyzer (LECO–TGA 701, USA).

Optimization of Ultrasound-Assisted Alkaline Pretreatment for Xylan Extraction

The design expert software (version 10.0.0, Stat-Ease Inc, Minneapolis, MN, USA) was used to optimize the ultrasound-assisted alkaline pretreatment condition for the extraction of xylan. Box–Behnken design was used to determine the effect of alkaline concentration (X_1), ultrasonication time (X_2) and solute-to-alkali solution ratio (X_3) on the extraction of xylan from sugarcane bagasse. Xylan recovery % (w/w) was determined as the response variable. The experiments were carried out in a randomized manner and the data was analyzed using a quadratic polynomial regression model as shown in Eq. (1).

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j \quad (1)$$

where Y is the response variable, β_0 is constant, β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients determined by the model and X_i and X_j are the independent variables, respectively.

Following the set of experimental design as obtained from Box–Behnken design, the sugarcane bagasse sample (2.5 g) was added to NaOH solution (0.0–1.0 M) at different solute-to-alkali solution ratios (1:10–1:40) and exposed to ultrasonication using an ultrasonic probe reactor (UP 200S, Hielscher, Teltow, Germany) for different time intervals (10–40 min) at a fixed frequency of 24 kHz.

Determination of Xylan Concentration

The xylan from ultrasonicated alkaline pretreated slurry was extracted following Samanta et al. (2012) with slight modifications. The slurry obtained after the ultrasonication was filtered through Whatman filter paper No. 1 and the filtrate containing xylan was adjusted to pH 5.4 with acetic acid (2 M). Precipitate was separated by centrifugation at $6440 \times g$ for 20 min (Hettich, EBA 8S). Pellets of the precipitate were then oven-dried at 60 °C until constant weight. The percentage true recovery of xylan from the bagasse was calculated using Eq. (2).

$$\text{True Recovery } \% = \frac{\text{Dry weight of extracted xylan (g)}}{\text{Weight of the sample (g)}} \times 100 \quad (2)$$

FTIR Analysis

Xylan extracted from the optimized condition and untreated sugarcane bagasse powder were subjected to FTIR analysis for characterization of the chemical structure. The Fourier transform infrared (FTIR) spectroscopy was performed by using a FTIR spectrometer (PerkinElmer, USA) with a 4 cm^{-1} resolution in the range of 600–4000 cm^{-1}. The sample together with potassium bromide (KBr) was ground to a fine powder. The mixture was transferred to the compression die under high pressure to form the pellets. The pellets were examined in accordance with the E1252 (2013) standard.

Enzymatic Hydrolysis of Extracted Xylan

Xylan extracted from the optimized pretreatment condition was hydrolyzed using the enzyme β-1,4-xylanase following the method as described by Akpinar et al. (2009). Xylan hydrolysis was done by incubating mixture of xylan (2% dissolved in sodium acetate buffer 50 mM, pH 5.4) and xylanase enzyme at different concentrations (10 U, 30 U, 50 U, 100 U, 200 U, 400 U and 800 U per g xylan) in an orbital shaker (50 °C at 250 rpm). Sample was taken at regular intervals (0–72 h) for xylose analysis.

Determination of Xylose Content

Sample (1 mL) was withdrawn from incubated hydrolysates slurry at different time intervals (0, 1, 2, 4, 8, 16, 24, 48, 60 and 72 h), heated (95 °C for 15 min) to inactivate the enzyme, followed by cooling to ambient temperature (25 °C). The sample was then centrifuged ($4025 \times g$, 20 min) and filtered through Whatman filter paper no. 1 to get the clear filtrate rich in xylose. Reducing sugar (xylose) concentration in the filtrate was determined by the Dinitro salicylic acid (DNS) method by following the method as described by Akpinar et al. (2009) with slight modifications. Xylose solution (0.1 mL) was added to DNS reagent (0.1 mL), vortex mixed and incubated in the hot water bath (95 °C for 15 min) until the appearance of red brown color. The mixture was cooled (25 °C) and distilled water (8 mL) was added to the mixture and vortex mixed. Absorbance of this solution was noted at 540 nm by using the UV–Vis spectrophotometer (UNICAM, Alva, UK). Xylose standard curve was obtained by using different concentrations of a standard xylose solution (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mg/mL). Xylose concentration (mg/mL) was calculated by using the equation ($A_{540\text{nm}} = 0.6055x - 00047$, $R^2 = 0.9989$) obtained from the xylose standard calibration curve (data in Supplementary File, Figure S1).

Microorganisms and Inoculum Preparation

The microbial samples of yeast and bacteria were prepared by following the method as described by Hernández-Pérez et al. (2016) and Yoshitake et al. (1971). Dried yeast, *C. guilliermondii* was rehydrated in yeast malt (YM) extract broth and streaked on the YM agar plate. Similarly, dried bacteria, *C. glutamicum* were rehydrated in nutrient broth and streaked on the nutrient agar (NA) plate. The plates were incubated at 30 °C for 48 h for the growth of desired colonies. The yeast cells were transferred to yeast propagation medium (50 mL) composed of xylose (3%), rice bran extract (4%), $(NH_4)_2SO_4$ (0.2%) and $CaCl_2{\cdot}2H_2O$ (0.01%). Bacterial cells were transferred to bacterial propagation medium (100 mL, pH 6.5) containing potassium gluconate (2.4%), $(NH_4)_2SO_4$ (0.2%), KH_2PO_4 (0.1%), $MgSO_4.7H_2O$ (0.05%), thiamine hydrochloride (20 µg/L) and $FeSO_4.7H_2O$ (5000 µg/L). The propagation cultures were subjected to rotary shaker (150 rpm) at 30 °C for 48 h. Afterward, the cells were separated by centrifugation at $4025 \times g$ for 20 min, rinsed twice with normal saline and the cell pellet was resuspended in normal saline and used as an inoculum.

Fermentation of Xylose-Rich Sugarcane Bagasse Hydrolysates

Sugarcane bagasse hydrolysate rich in xylose obtained from enzymatic hydrolysis was vacuum concentrated at 40 °C to increase the xylose concentration to 0.82% (w/v). The hydrolysate was subjected to microbial fermentation utilizing *C. guilliermondii*, *C. glutamicum* and their mixed culture.

Fermentation using *C. guilliermondii,* was conducted by following the method as described by Hernández-Pérez et al. (2016). Sugarcane bagasse hydrolysates (30 mL at pH 5.5) was inoculated with initial cell biomass concentration of 1 g/L in 125 mL cotton plugged Erlenmeyer flask and incubated

in an orbital shaker at 30 °C at 100 rpm. Fermentation additives used were same as that used in culture propagation medium except xylose. *C. glutamicum* fermentation was done by the method as described by Yoshitake et al. (1971). The bacterial inoculum maintained at cell concentration of 1 g/L was inoculated in gluconate medium (20 mL, pH 6.5) composed of potassium gluconate (9.6%), $(NH_4)_2SO_4$ (0.6%), KH_2PO_4 (0.1%), $MgSO_4.7H_2O$ (0.05%), thiamine hydrochloride (200 µg/L) and $FeSO_4.7H_2O$ (5 ppm) and incubated in the rotary shaker operating at 200 rpm at 30 °C for 2 days. After 2 days, 10 mL of sugarcane bagasse hydrolysates with xylose concentration (24.7 mg/mL) was aseptically transferred into the gluconate medium so that the final xylose concentration in the fermenting substrate was 0.82% (w/v). For the mixed culture fermentation, sugarcane bagasse hydrolysates (30 mL, pH 6.0) were inoculated with equal proportion of bacterial and yeast inoculum suspension to maintain final gross cell concentration of 1 g/L. All the fermentation additives used previously for *C. guilliermondii* and *C. glutamicum* culture were added. Finally, all the fermentation flasks with three different starter cultures were agitated in an orbital shaker at 30 °C at 100 rpm for a period up to 96 h.

Determination of Xylitol

The xylitol concentration was determined by using D-sorbitol/xylitol assay kit by following the manufacturer's instructions (Megazyme, Ireland). For confirmatory analysis of xylitol concentration, sample from each starter culture condition that yielded the highest concentrations of xylitol was analyzed by high-performance liquid chromatography (HPLC, Dionex UltiMate 3000, Illinois, USA) (Data in Supplementary File, Figure S2) following the method as described by Misra et al. (2011). HPLC was equipped with a refractive index detector (RID) and a Sugar Pak I Column (300 mm × 6.5 mm). Distilled water was used as a mobile phase with flow rate set as 0.5 mL/min, temperature at 90 °C and sample injection volume 20.0 µl.

Fermentation Parameters

For each fermentation, periodic sampling was done at an interval of 24, 48, 72 and 96 h to determine the xylose uptakes and xylitol production. Xylose uptake was calculated by DNS method and xylitol concentration was analyzed by D-xylitol/sorbitol assay kit and later reassured by HPLC for optimum fermentation condition of each culture strains. Yeast cell count was measured by hemocytometer, whereas bacterial and mixed culture growth was monitored by measuring optical density at 600 nm. Xylose consumption (%), xylitol yield (Yp/s) and xylitol recovery (%) were calculated using the following equations:

$$\text{Xylose consumption}\,(\%) = \left(\frac{\text{Initial xylose concentraion} - \text{Final xylose concentration}}{\text{Intial xylose concentration}}\right) \times 100 \tag{3}$$

$$\text{Xylitol yield}\left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{xylitol concentration in the broth at the end of fermentation}}{\text{Initial xylose concentration} - \text{Final xylose concentraion}} \tag{4}$$

$$\text{Xylitol recovery}\,(\%) = \left(\frac{\text{Xylitol concentration in the broth at the end of fermentation}}{\text{Mass of xylan incurred for xylitol formation}}\right) \times 100 \tag{5}$$

Partial Purification and Characterization of Xylitol

Xylitol obtained from microbial fermentation was partially purified following the method as described by Misra et al. (2011). Fermentation broth containing xylitol was centrifuged (4025×*g*, 20 min). The sediment containing cell biomass was discarded while the supernatant was treated with the activated charcoal (15 g/L) with a magnetic stirrer (30 °C for 1 h) and filtered through Whatman's filter paper No.1. The filtrate containing xylitol was freeze-dried and was further characterized by Fourier transform infrared (FTIR) spectroscopy as discussed in Sect. 2.5. The spectra of pure standard xylose and xylitol were also recorded by FTIR.

Statistical Analysis

Analysis of variance (ANOVA) was used to carry out the statistical analysis of response surface model (RSM) results to show the significance of the model and independent variables. One-way ANOVA was performed by using SPSS statistical software package (SPSS, version 23.0, USA). The significant differences between the mean values of different treatments at 95% confidence level ($p < 0.05$) were calculated by using LSD and Tukey's test.

Results and Discussion

Proximate and Compositional Analysis of Sugarcane Bagasse Powder

The proximate analysis of sugarcane bagasse powder showed 6.2% moisture, 78.46% volatile matter, 13.16% fixed carbon and 2.18% ash content. Hassuani (2005)

reported the proximate composition of bagasse as moisture content (50.2% w/w), volatile matter (79.9% w/w), fixed carbon (18% w/w) and ash (2.2% w/w). The moisture content of the bagasse used in this research was lower as it was oven-dried at 60 °C for 24 h. Further, compositional analysis showed that cellulose (49.38%) was the major component of sugarcane bagasse followed by hemicellulose (26.43%), lignin (12.85%), extractives (sucrose, nitrate/nitrite, protein, ash, chlorophyll, waxes) (9.13%). de Moraes Rocha et al. (2015) reported the average composition of sugarcane bagasse as cellulose (42.19%), hemicellulose (27.6%), lignin (21.56%), extractives (5.63%) and ash (2.84%).

Optimization of Ultrasound-Assisted Alkaline Pretreatment for Xylan Recovery

To optimize the ultrasound-assisted alkaline pretreatment for xylan recovery from sugarcane bagasse, response surface methodology was utilized. Box–Behnken design along with the experimental and predicted values is shown in Table 1.

A series of three-dimensional (3-D) response surface graphs were generated in which one variable was kept constant and the interactive effects of other two independent variables on xylan recovery was studied (Fig. 1). The 3-D graphs clearly indicated that NaOH concentration and solid–liquid ratio had a profound effect on xylan recovery such that xylan recovery increased with the increase in alkali concentration and solid-to-liquid ratio (Fig. 1a). The effect of combination of alkali concentration–ultrasonication time and solid–liquid ratio–ultrasonication time was observed to be nonsignificant on the response variable (Fig. 1b, c).

The increase in xylan recovery with the increase in alkali concentration was attributed to the ability of alkali to dissolve hemicellulose and lignin by hydrolysis of uronic and acetic esters and swelling up of cellulose, leading to the reduction in cellulose crystallinity (Peng et al. 2009). Jayapal et al. (2013) reported that sodium hydroxide concentration when increased from 2 to 12% resulted an increase in true recovery of xylan (sugarcane bagasse) from 2.77 to 19.88% (w/w).

Xylan recovery increased significantly ($p < 0.05$) with the increase in solid–liquid ratio which works based on the principle of mass transfer, i.e., xylan concentration gradient was higher in the bagasse than in the solvent (alkali) that acts as a driving force for mass transfer from solid to solvent. With the increase in alkali ratio, concentration gradient also increases accordingly and as the equilibrium concentration is reached, mass transfer stops (Vinatoru et al. 2017). Similarly, Sun et al. (2015) reported increase in xylan extraction efficiency from corncob with the increase in corncob to alkali solution ratio from 1:10 to 1:30.

Ultrasonication invades and disrupts the cell wall integrity by creating a cavitation phenomenon followed by the generation of microbubbles and shear forces along with cleaving of the ether bonds present between the

Table 1 Box–Behnken experimental design with predicted and actual values of xylan recovery

Run order	Independent variables			Response variable (xylan recovery %)	
	NaOH concentration (M) (X_1)	Solid–liquid (g:mL) (X_2)	UAE time (min) (X_3)	Predicted	Experimental
1	0.5	40	10	9.81	10.21
2	0	10	25	0.11	0.56
3	1	40	25	11.70	11.25
4	0.5	10	10	7.99	7.71
5	0.5	25	25	10.02	10.19
6	1	25	10	10.10	10.15
7	0	25	40	0.78	0.73
8	1	10	25	8.35	8.57
9	0.5	10	40	7.86	7.46
10	0	25	10	0.97	0.80
11	1	25	40	10.61	10.78
12	0.5	25	25	10.02	10.20
13	0	40	25	1.00	0.77
14	0.5	40	40	10.27	10.56
15	0.5	25	25	10.02	9.67

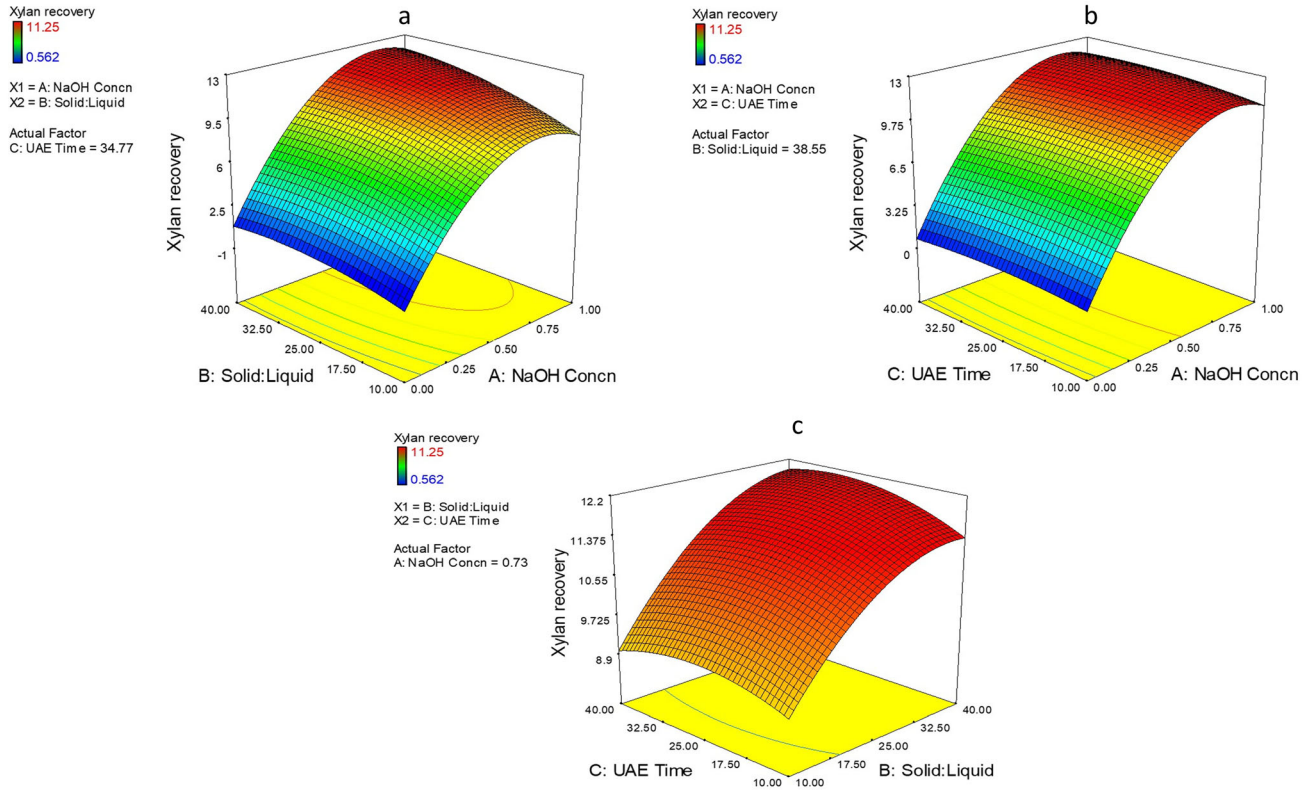

Fig. 1 Response surface 3D graph (**a**–**c**) showing interactive effects of NaOH concentration, solid-to-liquid ratio and ultrasonication time on xylan recovery %

hemicellulose and lignin, resulting in increased accessibility and extractability of xylan (Sun et al. 2004). In the present study, although an increase in ultrasonication time (from 10 to 40 min) showed a positive effect on xylan recovery, the recovery was not significantly increased ($p > 0.05$) with the extension of time. This was due to the effect of ultrasonication, which led to a maximum recovery of xylan in a very short time. During the two-step extraction procedure of heteroxylan from wheat bran, Hromádková et al. (2008) reported that the use of short time (up to 10 min) ultrasound-assisted extraction (UAE) reduced the extraction time by 60% as compared to conventional extraction process (without ultrasound) that lasted for 60 min.

Based on the desirability function of Design Expert, the experimental xylan recovery value (11.89% w/w) was observed to be similar to predicted maximum xylan recovery (12.059%) at NaOH concentration (0.73 M), ultrasonication time (34.77) min and solid-to-liquid ratio (1:38.55), hence conforming its validity. Jayapal et al. (2013) reported that sugarcane bagasse steamed in 1 M NaOH solution at a solid–liquid ratio of 1:10 resulted in lower xylan recovery of 6.11% w/w. Therefore, xylan recovery from sugarcane bagasse can be maximized by optimizing alkaline pretreatment condition accompanied with ultrasound treatment.

FTIR Analysis

The effect of combined alkaline–ultrasonication pretreatment on the chemical structure of xylan was studied by the FITR spectroscopy (Fig. 2). The functional groups of the compounds were identified by comparing the obtained FTIR spectrum data with the reference data from Coates (2006). The protruding broad absorption band at 3410.20 and 3404.11 cm^{-1} of bagasse powder and xylan, respectively, depicted the stretching of hydroxyl (–OH) groups. Development of distinct absorbance bands in xylan spectra at 3410.20, 1414.45, 1043.88 and 897.30 cm^{-1} were associated with the presence of xylan as reported by Jayapal et al. (2013). The spectrum between 1160.45 and 1043.88 cm^{-1} was also associated with the typical xylan molecule. The absence of peak in xylan at around 1730 cm^{-1} indicated the complete cleavage of ester bonds of xylan molecule due to the ultrasonic and alkaline pretreatment (Brienzo et al. 2009).

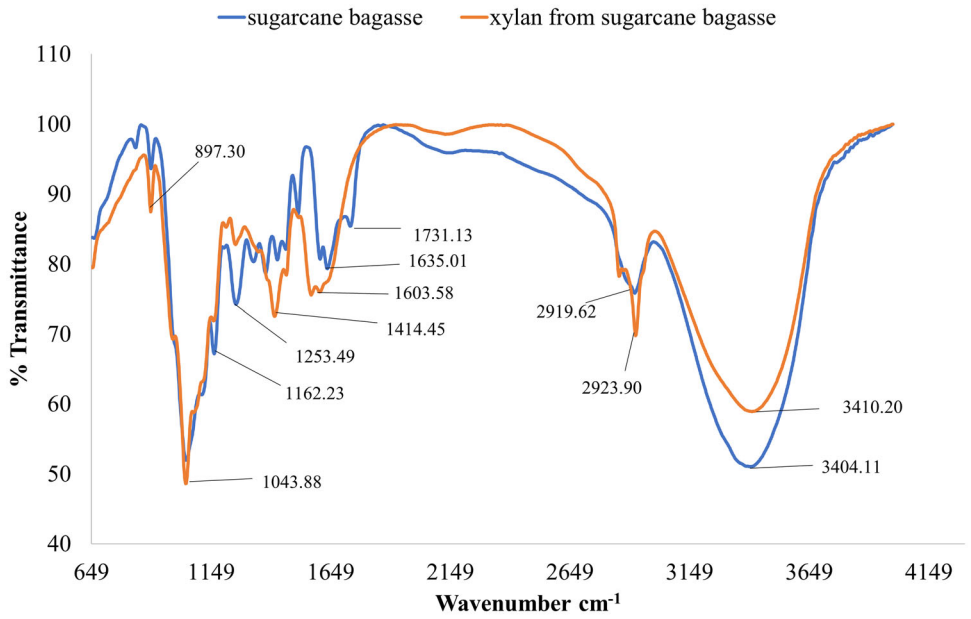

Fig. 2 Comparison of FTIR spectra of xylan and sugarcane bagasse powder

Enzymatic Hydrolysis of Xylan to Xylose

The effect of β-1,4-xylanase enzyme at different concentration and hydrolysis time on the production of xylose was studied (Fig. 3). Statistical analysis illustrated that at each enzyme concentration, the xylose production increased significantly ($p < 0.05$) up to 48 h and the increasing trend plateaued thereafter. Hence the time, 48 h was selected as the optimum enzymatic hydrolysis time. Further, at each incubation time, significant increment ($p < 0.05$) in xylose concentration was reported with the increase in enzyme concentration from 10 to 400 U/g, above which (800 U/g) no significant difference ($p < 0.05$) was observed. Therefore, enzyme concentration of 400 U/g xylan was determined as the optimum enzyme dosage. Overall, optimum enzymatic hydrolysis condition was achieved at an enzyme dosage of 400 U/g xylan at 48 h of incubation time with xylose yield of 81.51 ± 1.73 mg/g and 30.84% of total xylose recovery.

The increase in xylose conversion with the increase in enzyme concentration and incubation time can be attributed to the proportional relationship of enzymatic reaction rate and corresponding xylose yield with the concentration of enzyme loaded and time of hydrolysis. Damaso et al.

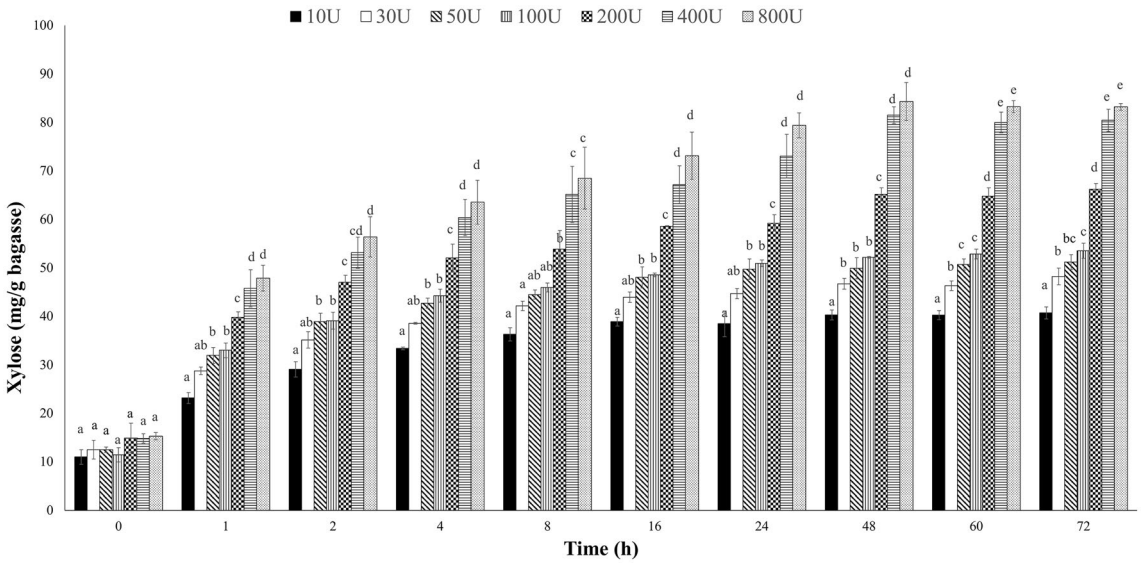

Fig. 3 Effect of different enzyme concentrations on xylose recovery at different hydrolysis period. Different superscript letters (a–e) indicate significant difference ($p < 0.05$) between different enzyme dosage rates (10–800 U/g) at a particular time

(2004) reported the enzymatic hydrolysis of alkaline (2 N NaOH) and thermally pretreated sugarcane bagasse using 3000U/g crude xylanase for 24 h which resulted in xylose yield of 25.2 mg/g bagasse (9.52% of total xylose recovery) which was lower as compared to the present study. Brienzo et al. (2010) reported that the optimized xylanase concentration of 120 U/g was found to produce 17.98% of total xylose from sugarcane bagasse. Paiva et al. (2009) reported that acid hydrolysis of sugarcane bagasse with sulfuric acid (3.1% v/v) at 126 °C for 18 min of reaction time produced xylose (266.73 mg/g bagasse) which was equivalent to more than 96% of the theoretical yield. However, acid hydrolysates contain various degradation toxic and fermentation inhibitory compounds (furfural, acetic acids, HMFs and LDPs) that requires detoxification step prior to fermentation, whereas enzymatic hydrolysates are devoid of these toxic compounds (Mohamad et al. 2015).

Fermentation of Xylose into Xylitol by Different Cultures

The sugarcane bagasse hydrolysates rich in xylose obtained by enzymatic hydrolysis were inoculated with three different starter cultures; *C. guilliermondii*, *Corynebacterium glutamicum* and their mixed culture to produce xylitol. Figure 4 illustrates the profile of substrate (xylose) consumption and product (xylitol) formation by respective three starter culture strains in a batch culture for a period of 96 h. Initial xylose concentration of 8.25 g/L was maintained at all culture conditions. At the end of fermentation (72 h), maximum xylose consumption resulted by yeast (67.71%), followed by mixed culture (48.70%) and bacteria (25.76%). Similarly, maximum xylitol concentration was accumulated at 72 h of fermentation by yeast (2.39 ± 0.13 g/L) followed by mixed culture (1.53 ± 0.09 g/L) and bacteria (0.26 ± 0.03 g/L), respectively.

At all culture conditions, xylitol concentration and xylitol yield increased significantly ($p < 0.05$) with the increase in fermentation time until 72 h followed by significant ($p < 0.05$) decrement at 96 h. Xylose concentration in the fermentation broth decreased continuously which indicated xylose conversion into xylitol upon its consumption by the microbial cells for their growth and metabolism. Observance of maximum xylitol accumulation at 72 h followed by decrement in xylitol concentration at 96 h was due to the fact that yeast cells start xylitol utilization for further metabolism when the xylose in the broth is in the exhausting phase (Prakash et al. 2011). Vallejos et al. (2016) obtained 0.99 g/L of xylitol by *C. guilliermondii* with an initial concentration of 30 g/L of xylose after 96 h of fermentation, which was lower than the current study. Xylitol (an intermediate metabolite of xylose metabolism) conversion involves two steps, a reduction process followed by an oxidation process. At first, D-xylose is reduced to D-xylitol by NADPH, and then, this metabolite is oxidized to D-xylulose by NADP+, resulting these two reactions to be a limiting factor for D-xylose fermentation and D-xylitol production (Albuquerque et al. 2014). In case of slight fluctuations in oxygen availability, xylitol formation pathway enters pentose phosphate pathway preventing the xylitol accumulation and similarly, xylitol formation is indispensably associated with the formation of other metabolites like ethanol, carbon dioxide, acetic acid and polysaccharides, thereby resulting lower xylitol conversion rate (Rafiqul and Sakinah 2013). The reported variations in the values of xylitol are associated

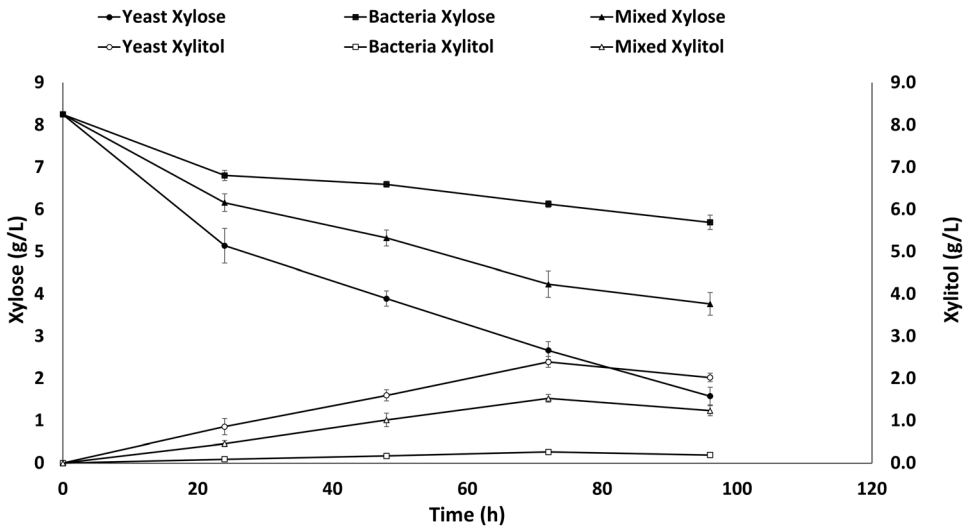

Fig. 4 Xylitol formation and xylose consumption (black symbol) at different time periods during fermentation of sugarcane bagasse hydrolysates by *Candida guilliermondii*, *Corynebacterium glutamicum* and their mixed culture

with different microbial species and varying growth conditions involved.

Fermentation Parameters

The effect of three different starter cultures on xylitol production was evaluated based on xylose consumption, xylitol yield and xylitol recovery (%) (Table 2). Yeast strain *C. guilliermondii* showed the highest xylitol yield (Yp/s = 0.43 g/g) and xylitol recovery % (8.97% w/w of total xylan used) compared to bacteria (Yp/s = 0.13 g/g) and mixed culture (Yp/s = 0.38 g/g) that confirmed *C. guilliermondii* as the suitable starter culture for xylitol production. However, the xylitol yield from *C. guilliermondii* was observed to be comparatively lower than those reported previously; da Cunha et al (2009) reported the final xylitol yield of 0.49 g/g by using PVA-hydrogel entrapped yeast whereas de Arruda et al. (2017) reported the xylitol yield of 0.55 g/g while scaling up to pilot scale. Palladino et al. (2021) reported a xylitol yield of 0.63 g/g by using the novel yeast strain *C. xylosilytica* UFMG-CM-Y-309, which was slightly higher than this study. Vallejos et al. (2016) reported a maximum xylitol yield of 0.46 g/g by using *C. tropicalis*. This comparative lower yield can be attributed to difference in fermentation parameters and the presence of divergent and inhibitors in the fermenting substrate as fermentation is associated with the microbial tolerances level and growth conditions (Vallejos et al. 2016).

For the mixed culture, values for these parameters were lower than that of yeast fermentation and higher than that of bacterial fermentation. This implies that in mixed culture, xylitol formation efficiency of bacteria was enhanced, whereas that of yeast was reduced. This phenomenon of low product yield can also be attributed to the partial inhibitory effect on each other. Coculture and mixed fermentation exhibits various interactions like competition, commensalism, predation, mutualism and proto cooperation between microbial communities (Shimizu et al. 2005). Similarly, various process parameters; pH, temperature, oxygen, substrate and product concentration are responsible for achieving the synergistic effect of mixed and coculture cultivation (Bader et al. 2010).

Partial Purification of Xylitol and Characterization by FTIR

Partially purified freeze-dried xylitol obtained from the fermentation was subjected to FTIR analysis which was compared with the spectra obtained for standard xylitol. Pure xylose spectra were used to explain the peaks obtained in freeze-dried xylitol due to the presence of residual sugars. The functional groups were identified by comparing the obtained spectrum data with the reference data from Coates (2006). The spectra of freeze-dried xylitol, pure xylitol and xylose are presented in Fig. 5.

IR spectra of freeze-dried xylitol showed a broad stretch around 3389.98 cm^{-1} which was similar to the stretch of pure xylitol spectra that ranged from 3427.77 to 3190.12 cm^{-1} corresponding to the chemical characteristic of hydroxyl group (–OH) present in five carbon sugar alcohol. A weak bond of C–H stretching band was also observed for both freeze-dried and pure xylitol at around 2938.80 cm^{-1} and at a range of 2995.75–2879.71 cm^{-1}, respectively. Pure xylitol spectra showed the distinct peak 1420.21 cm^{-1} and in the similar manner, freeze-dried xylitol also showed sharp peak at 1412.86 cm^{-1} corresponding to the O–H bending of carboxylic acid. This resembles to a previous study by Mukherji et al. (2013) who reported the presence of strong typical peak at around 1410 cm^{-1} and 2931 cm^{-1} during the IR spectral analysis of pure xylitol and xylitol crystal obtained from fermentation broth similar to current study. A very sharp distinct band was observed at 1041.10 cm^{-1} in pure xylose spectra and similar to this peak, a sharp peak at 1046.45 cm^{-1} was observed in freeze-dried xylitol which indicated the presence of residual sugars (xylose) in the sample. However, a strong sharp bend of N–H was observed in freeze-dried xylitol at 1567.46 cm^{-1} corresponding to secondary amine group which was not observed in pure xylitol spectra.

Table 2 Comparison of xylose consumption, xylitol yield and xylan recovery by different starter cultures at 72 h of fermentation

Starter culture	Xylose consumption (%)	Xylitol concentration (g/L)	Xylitol yield, Yp/s (g/g)	Xylitol recovery of total xylan (%)
C. guilliermondii	67.71 ± 2.52^{c}	2.39 ± 0.13^{c}	0.43 ± 0.02^{c}	8.97 ± 0.48^{c}
Corynebacterium glutamicum	25.76 ± 0.95^{a}	0.26 ± 0.03^{a}	0.13 ± 0.02^{a}	0.99 ± 0.10^{a}
C. guilliermondii and *Corynebacterium glutamicum*	48.70 ± 3.79^{b}	1.53 ± 0.09^{b}	0.38 ± 0.02^{b}	5.73 ± 0.34^{b}

Different superscript letters (a–c) within a column indicate significant differences ($p < 0.05$) between mean observations

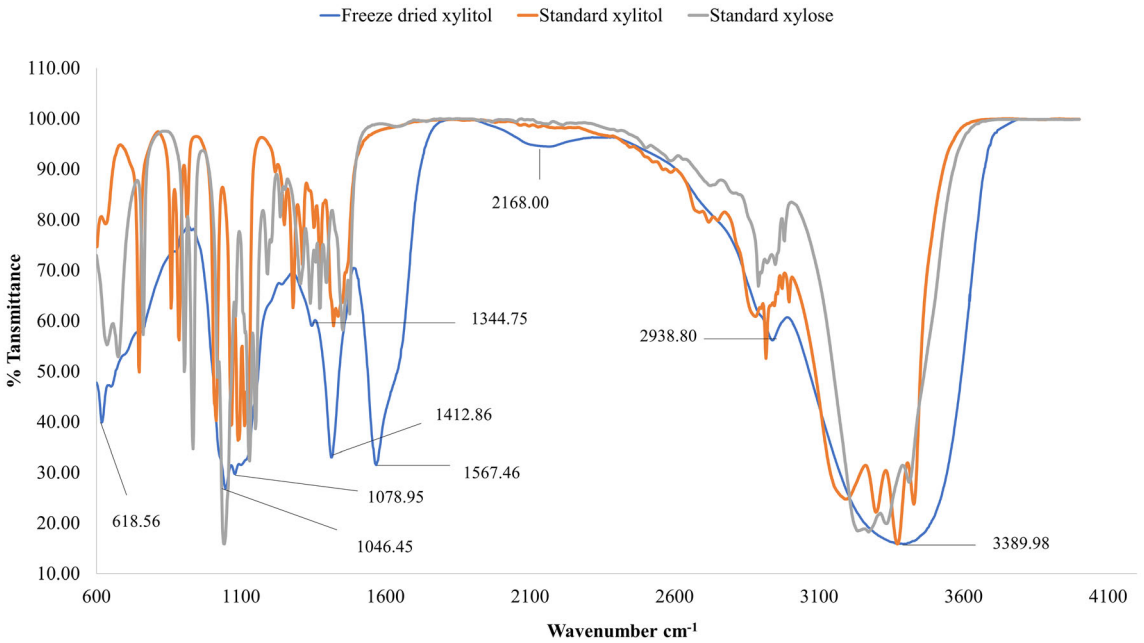

Fig. 5 FTIR spectra of partially purified freeze-dried xylitol, pure xylitol and xylose standard

Hence, based on the common spectral peaks between pure xylitol and freeze-dried xylitol and resemblance with xylose structure, the partially purified compound was confirmed to be xylitol with some residual sugars (xylose).

Conclusion

The present study demonstrated a biotechnological processing for xylitol production from sugarcane bagasse hydrolysates rich in xylose. It was revealed that sugarcane bagasse can be hydrolyzed by a biobased enzymatic hydrolysis associated with ultrasound-assisted mild alkaline pretreatment and consequently, as a substitute to acid hydrolysis, the most common and conventional hydrolysis method of agroindustrial waste. Ultrasound-assisted alkaline pretreatment was found to increase the hydrolysis efficiency of xylanase. Furthermore, this study confirmed that yeast (*C. guilliermondii*) was the best starter culture for xylitol production from sugarcane bagasse hydrolysates as compared to bacteria (*Corynebacterium glutamicum*) and the mixed culture (*C. guilliermondii & Corynebacterium glutamicum*). Overall, this study provides a suitable alternative for industrial utilization of hemicellulose-rich sugarcane bagasse for xylitol production.

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Code Availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics Approval Not applicable.

Consent for Publication Not applicable.

Consent to Participate Not applicable.

References

Akpinar, O., K. Erdogan, and S. Bostanci. 2009. Enzymatic production of Xylooligosaccharide from selected agricultural wastes. *Food and Bioproducts Processing* 87 (2): 145–151. https://doi.org/10.1016/j.fbp.2008.09.002.

ASTM E1252-98. 2013. *Standard practice for general techniques for obtaining infrared spectra for qualitative analysis*. West Conshohocken, PA: ASTM International. https://doi.org/10.1520/E1252-98R13E01.

Bader, J., E. Mast-Gerlach, M.K. Popović, R. Bajpai, and U. Stahl. 2010. Relevance of microbial coculture fermentations in biotechnology. *Journal of Applied Microbiology* 109 (2): 371–387. https://doi.org/10.1111/j.1365-2672.2009.04659.x.

Brienzo, M., W. Carvalho, and A.M. Milagres. 2010. Xylooligosaccharides production from alkali-pretreated sugarcane bagasse using xylanases from *Thermoascus aurantiacus*. *Applied Biochemistry and Biotechnology* 162 (4): 1195–1205. https://doi.org/10.1007/s12010-009-8892-5.

Brienzo, M., A.F. Siqueira, and A.M.F. Milagres. 2009. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. *Biochemical Engineering Journal* 46 (2): 199–204. https://doi.org/10.1016/j.bej.2009.05.012.

Cardona, C.A., J.A. Quintero, and I.C. Paz. 2010. Production of bioethanol from sugarcane bagasse: Status and perspectives. *Bioresource Technology* 101 (13): 4754–4766. https://doi.org/10.1016/j.biortech.2009.10.097.

Carrier, M., A. Loppinet-serani, D. Denux, and J. Lasnier. 2011. Thermogravimetric analysis as a new method to determine the lignocellulosic composition of biomass. *Biomass and Bioenergy* 35 (1): 298–3075. https://doi.org/10.1016/j.biombioe.2010.08.067.

Chandel, A.K., S.S. da Silva, W. Carvalho, and O.V. Singh. 2012. Sugarcane bagasse and leaves: Foreseeable biomass of biofuel and bio-products. *Journal of Chemical Technology and Biotechnology* 87 (1): 11–20. https://doi.org/10.1002/jctb.2742.

Chen, X., Z. Jiang, S. Chen, and W. Qin. 2010. Microbial and bioconversion production of D-xylitol and its detection and application. *International Journal of Biological Sciences* 6 (7): 834–844.

Coates, J. 2006. *Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry: applications, theory and instrumentation*, 1–23. Chichester: John Wiley & Sons Ltd. https://doi.org/10.1002/9780470027318.a5606.

da Cunha, M.A., A. Converti, J.C. Santos, S.T. Ferreira, and S.S. da Silva. 2009. PVA-hydrogel entrapped *Candida guilliermondii* for xylitol production from sugarcane hemicellulose hydrolysate. *Applied Biochemistry and Biotechnology* 157 (3): 527–537. https://doi.org/10.1007/s12010-008-8301-5.

Damaso, M. C. T., A. M. De Castro, R. M. Castro, C. M. M. Andrade, and N. Pereira. 2004. Application of xylanase from *Thermomyces lanuginosus* IOC-4145 for enzymatic hydrolysis of corncob and sugarcane bagasse. In *Proceedings of the twenty-fifth symposium on biotechnology for fuels and chemicals held May 4–7, 2003, in Breckenridge, CO*, 1003–1012. Totowa, NJ: Humana Press.

Dasgupta, D., S. Bandhu, D.K. Adhikari, and D. Ghosh. 2017. Challenges and prospects of xylitol production with whole cell bio-catalysis: A review. *Microbiological Research* 197: 9–21. https://doi.org/10.1016/j.micres.2016.12.012.

De Albuquerque, T.L., I.J. Da Silva, G.R. De MacEdo, and M.V.P. Rocha. 2014. Biotechnological production of xylitol from lignocellulosic wastes: A review. *Process Biochemistry* 49 (11): 1779–1789. https://doi.org/10.1016/j.procbio.2014.07.010.

de Arruda, P.V., J.C. dos Santos, R.D.C.L.B. Rodrigues, D.D.V. da Silva, C.K. Yamakawa, G.J. de Moraes Rocha, and M.D.G. de Almeida Felipe. 2017. Scale up of xylitol production from sugarcane bagasse hemicellulosic hydrolysate by *Candida guilliermondii* FTI 20037. *Journal of Industrial and Engineering Chemistry* 47: 297–302. https://doi.org/10.1016/j.jiec.2016.11.046.

de Moraes Rocha, G.J., V.M. Nascimento, A.R. Goncalves, V.F.N. Silva, and C. Martín. 2015. Influence of mixed sugarcane bagasse samples evaluated by elemental and physical–chemical composition. *Industrial Crops and Products* 64: 52–58. https://doi.org/10.1016/j.indcrop.2014.11.003.

Elamin, K., J. Sjöström, H. Jansson, and J. Swenson. 2012. Calorimetric and relaxation properties of xylitol-water mixtures. *Journal of Chemical Physics* 136 (10): 104508. https://doi.org/10.1063/1.3692609.

Hernández-Pérez, A.F., I.A.L. Costa, D.D.V. Silva, K.J. Dussán, T.R. Villela, E.V. Canettieri, J.A. Carvalho Jr., T.G. Soares Neto, and M.G.A. Felipe. 2016. Biochemical conversion of sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates for xylitol production. *Bioresource Technology* 200: 1085–1088. https://doi.org/10.1016/j.biortech.2015.11.036.

Hromádková, Z., Z. Košt'Álová, and A. Ebringerová. 2008. Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. *Ultrasonics Sonochemistry* 15 (6): 1062–1068. https://doi.org/10.1016/j.ultsonch.2008.04.008.

Hassuani, S.J., M.R.L.V. Leal, and I. de Carvalho Macedo, eds. 2005. *Biomass power generation: sugar cane bagasse and trash*. CTC.

Jayapal, N., A.K. Samanta, A.P. Kolte, S. Senani, M. Sridhar, K.P. Suresh, and K.T. Sampath. 2013. Value addition to sugarcane bagasse: Xylan extraction and its process optimization for xylooligosaccharides production. *Industrial Crops and Products* 42: 14–24. https://doi.org/10.1016/j.indcrop.2012.05.019.

Martinez-hernandez, E., M. A. Amezcua-allieri, J. Sadhukhan, J. A. Anell, and M. A. Amezcua-allieri. 2018. *Sugarcane bagasse valorization strategies for bioethanol and energy production, sugarcane—technology and research*, Alexandre Bosco de Oliveira, IntechOpen. https://doi.org/10.5772/intechopen.72237.

Misra, S., P. Gupta, S. Raghuwanshi, K. Dutt, and R.K. Saxena. 2011. Comparative study on different strategies involved for xylitol purification from culture media fermented by *Candida tropicalis*. *Separation and Purification Technology* 78 (3): 266–273. https://doi.org/10.1016/j.seppur.2011.02.018.

Mohamad, N.L., S.M. Mustapa Kamal, and M.N. Mokhtar. 2015. Xylitol biological production: A review of recent studies. *Food Reviews International* 31 (1): 74–89. https://doi.org/10.1080/87559129.2014.961077.

Mukherji, R., K. Joshi-Navare, and A. Prabhune. 2013. Crystalline xylitol production by a novel yeast, *Pichia caribbica* (HQ222812), and its application for quorum sensing inhibition in Gram-negative marker strain *Chromobacterium violaceum* CV026. *Applied Biochemistry and Biotechnology* 169 (6): 1753–1763. https://doi.org/10.1007/s12010-012-0039-4.

Mussatto, S.I., and I.C. Roberto. 2002. Xilitol: Edulcorante com efeitos benéficos para a saúde humana. *Revista Brasileira De Ciências Farmacêuticas* 38: 401–413. https://doi.org/10.1590/S1516-93322002000400003.

Nabors, L.O.B., ed. 2001. *Alternative sweeteners.* 3rd ed., rev. expanded, vol. 112. In Food science and Technology. New York: M. Dekker. ISBN 978-0-8247-0437-7.

O'Donnell, K., and M. Kearsley, eds. 2012. *Sweeteners and sugar alternatives in food technology*. Oxford: Wiley.

Paiva, J.E.D., I.R. Maldonade, and A.R. Scamparini. 2009. Xylose production from sugarcane bagasse by surface response methodology. *Revista Brasileira De Engenharia Agrícola e Ambiental* 13: 75–80.

Palladino, F., R.C.L.B. Rodrigues, R.M. Cadete, K.O. Barros, and C.A. Rosa. 2021. Novel potential yeast strains for the biotechnological production of xylitol from sugarcane bagasse. *Biofuels, Bioproducts and Biorefining* 15 (3): 690–702. https://doi.org/10.1002/bbb.2196.

Peng, F., J.L. Ren, F. Xu, J. Bian, P. Peng, and R.C. Sun. 2009. Comparative study of hemicelluloses obtained by graded ethanol precipitation from sugarcane bagasse. *Journal of Agricultural and Food Chemistry* 57 (14): 6305–6317. https://doi.org/10.1021/jf900986b.

Prakash, G., A.J. Varma, A. Prabhune, Y. Shouche, and M. Rao. 2011. Microbial production of xylitol from D -xylose and sugarcane bagasse hemicellulose using newly isolated thermotolerant yeast *Debaryomyces hansenii*. *Bioresource Technology* 102 (3): 3304–3308. https://doi.org/10.1016/j.biortech.2010.10.074.

Rafiqul, I.S.M., and A.M.M. Sakinah. 2013. Processes for the production of xylitol—A review. *Food Reviews International* 29 (2): 127–156. https://doi.org/10.1080/87559129.2012.714434.

Rao, R.S., C.P. Jyothi, R.S. Prakasham, P.N. Sarma, and L.V. Rao. 2006. Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis*. *Bioresource Technology* 97 (15): 1974–1978. https://doi.org/10.1016/j.biortech.2005.08.015.

Samanta, A.K., N. Jayapal, A.P. Kolte, S. Senani, M. Sridhar, K.P. Suresh, and K. T. Sampath. 2012. Enzymatic production of xylooligosaccharides from alkali solubilized xylan of natural grass (*Sehima nervosum*). *Bioresource Technology* 112: 199–205. https://doi.org/10.1016/j.biortech.2012.02.036.

Shimizu, H., B. Cheirsilp, and S. Shioya. 2005. Development of co-culture systems of lactic acid bacteria and yeasts for bioproduction. *Japanese Journal of Lactic Acid Bacteria* 16 (1): 2–10.

Sun, J.X., R. Sun, X.F. Sun, and Y. Su. 2004. Fractional and physico-chemical characterization of hemicelluloses from ultrasonic irradiated sugarcane bagasse. *Carbohydrate Research* 339 (2): 291–300. https://doi.org/10.1016/j.carres.2003.10.027.

Sun, J., Z. Zhang, F. Xiao, and X. Jin. 2015. Production of xylooligosaccharides from corncobs using ultrasound-assisted enzymatic hydrolysis. *Food Science and Biotechnology* 24 (6): 2077–2081. https://doi.org/10.1007/s10068-015-0276-8.

Vallejos, M.E., M. Chade, E.B. Mereles, D.I. Bengoechea, J.G. Brizuela, F.E. Felissia, and M.C. Area. 2016. Strategies of detoxification and fermentation for biotechnological production of xylitol from sugarcane bagasse. *Industrial Crops and Products* 91: 161–169. https://doi.org/10.1016/j.indcrop.2016.07.007.

Velmurugan, R., and K. Muthukumar. 2012. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology. *Bioresource Technology* 112: 293–299. https://doi.org/10.1016/j.biortech.2012.01.168.

Vinatoru, M., T.J. Mason, and I. Calinescu. 2017. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. *TrAC - Trends in Analytical Chemistry* 97: 159–178. https://doi.org/10.1016/j.trac.2017.09.002.

Yoshitake, J., H. Ohiwa, M. Shimamura, and T. Imai. 1971. Production of polyalcohol by a *Corynebacterium* sp. Part I. Production of pentitol from aldopentose. *Agricultural and Biological Chemistry* 35 (6): 905–911. https://doi.org/10.1080/00021369.1971.10860014.

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