Evaluation of xylitol production using corncob hemicellulosic hydrolysate by combining tetrabutylammonium hydroxide extraction with dilute acid hydrolysis

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ABSTRACT

In this paper, we produced hemicellulosic hydrolysate from corncob by tetrabutylammonium hydroxide (TBAH) extraction and dilute acid hydrolysis combined, further evaluating the feasibility of the resultant corncob hemicellulosic hydrolysate used in xylitol production by Candida tropicalis. Optimized conditions for corncob hemicellulose extraction by TBAH was obtained via response surface methodology: time of 90 min, temperature of 60 °C, liquid/solid ratio of 12 (v/w), and TBAH concentration of 5%, resulting in a hemicellulose extraction of 80.07% under these conditions. The FT-IR spectrum of the extracted corncob hemicellulose is consistent with that of birchwood hemicellulose and exhibits specific absorbance of hemicelluloses at 1380, 1168, 1050, and 900 cm⁻¹. In addition, we found that C. tropicalis can ferment the resulting corncob hemicellulosic hydrolysate with pH adjustment and activated charcoal treatment leading to a high xylitol yield and productivity of 0.77 g/g and 2.45 g/(L·h), respectively.

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1. Introduction

Lignocellulosic materials are the most abundant renewable resources on the earth. For a long time, there has been an increasing interest in the use of lignocellulosic materials for industrial applications. As a major component of lignocellulosic materials, hemicellulose is a polysaccharide consisting commonly of xylose and it accounts for up to 40% of dry mass in some plants (Rao, Jyothi, Prakasham, Sarma, & Rao, 2006). Hemicellulose has been used as adhesives, thickeners and emulsifiers (Doner and Hicks, 1997). In particular, hemicellulose can also be hydrolyzed readily in dilute acid to generate hemicellulosic hydrolysates mostly comprised of xylose (Xu & Hanna, 2010). The hemicellulosic hydrolysates are promising starting material for many value-added products (Peng, Ren, Xu, & Sun, 2011).

Among those products derived from hemicellulosic hydrolysates, xylitol is a very important target. As we all know, xylitol is a commercial sweetener with high sweetening power and solubility, low calorie content, lack of carcinogenicity and cariostatic properties, which has been widely used as a replacement for sucrose in food industry (Aguirre-Zero, Zero, & Proskin, 1993; Lynch & Milgrom, 2003; Ronda, Gómez, Blanco, & Caballero, 2005).

Currently, xylitol production from hemicellulosic hydrolysates via benign biotechnological routes is attracting much attention. Many microorganisms have been evaluated for their capacity to convert hemicellulosic hydrolysates to xylitol under mild conditions (Mohamada, Mustapa, Kamala, & Mokhtara, 2015). Among them, yeasts are the best natural producers of xylitol (Chindea, Csaták, Stoica, Tanase, & Vassu, 2010). Substantial studies have shown great potential and interest of biotechnological production of xylitol from hemicellulosic hydrolysates by yeast (de Albuquerque, Gomes, Marques, da Silva, & Rocha, 2015).

For fulfilling the requirements of economically and environmentally friendly utilization of hemicelluloses, efficient extraction is an important prerequisite (Lan, Liu, & Sun, 2011). For now, various methods have been developed to extract hemicelluloses from lignocellulosic materials, such as alkali extraction, organic extraction, and hot water extraction (Shi et al., 2013). The resulting hemicelluloses extracted from different starting materials have been well characterized by chemical analysis, thermogravimetric analysis (TGA), ion-moderated partition chromatography (IMP), size exclusion chromatography (SEC) or gel permeation chromatography (GPC), Fourier transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance spectroscopy (NMR) (Buranov & Mazza, 2010; Luo et al., 2012; Zhang et al., 2016). For the purpose of efficient use of hemicelluloses, however, new environmentally benign pathways for extraction of hemicellulose with readily operation and low cost are still necessary to be developed.
Recently, tetrabutylammonium hydroxide (TBAH) and tetramethylammonium hydroxide (TMAH), two quaternary ammonium bases, have been confirmed to dissolve lignocelluloses under mild conditions in our previous reports (Zhong, Wang, Huang, Jia, & Wei, 2013; Zhong et al., 2016). Here, we have established a new protocol to extract hemicellulose from lignocellulosic materials by TBAH. Results showed that TBAH is a good solvent for hemicellulose extraction from lignocellulosic materials and it can be recycled in the processes.

In this study, we also optimized the process to extract hemicellulose from corncob by using TBAH to test the usability of TBAH in hemicellulose extraction. The resulting hemicellulose was characterized by FT-IR and hydrolyzed by dilute acid, and the resultant hydrolysate was used to evaluate the feasibility of xylitol production by fermentation of Candida tropicalis CICC1779.

2. Materials and methods

2.1. Materials

Corncob was purchased from Nantong, Jiangsu, China. Tetrabutylammonium hydroxide (55% aqueous solution) was provided by Alfa Aesar. Hemicellulose, xylene and xylitol were purchased from Aladdin Reagent Co. Ltd. All other reagents are of analytical grade unless otherwise noted.

2.2. Microorganism and medium

C. tropicalis CICC1779 was received from the China Center of Industrial Culture Collection (CICC). The medium for seed culture was prepared as follows (g/L): xylose 10, yeast extract 10, MgSO4 0.2, KH2PO4 5. Xylitol production was performed in a medium containing xylose with the following composition (g/L): yeast extract 2.5, peptone 2.5, KH2PO4 5, MgSO4 0.5, (NH4)2SO4 4.05.

2.3. Extraction of hemicellulose from corncob by TBAH

The scheme of hemicellulose extraction from corncob by TBAH was shown in Fig. 1. Hemicellulose extraction was carried out in a 250-ml flask. A certain amount (0.4–0.65 g) of corncob (100 mesh) was mixed with 5 mL of TBAH (50–60% aqueous solution). The mixtures were agitated at a certain temperature (50–70 °C) for a certain time (60–120 min) until the whole corncob was dissolved in the solution. Fifty milliliter of deionized water was then added and cellulose was precipitated. Subsequently, the mixtures were centrifuged at 5500 rpm, 4 °C for 3 min to collect the liquid fractions. Later, the liquid fractions were then adjusted to pH 4.0 using acetic acid and stored at 4 °C overnight for hemicellulose precipitation. Finally, the liquid fractions were centrifuged at 5500 rpm for 3 min to collect hemicellulose. The hemicellulose pellets were washed three times with 95% ethanol and air-dried.

2.4. Optimization of hemicellulose extraction by TBAH

In order to optimize hemicellulose extraction by TBAH, we carried out experiments based on a Box–Behnken design with the critical variables being the liquid/solid ratio (v/w) (X1, 8–12), temperature (X2, 50–70 °C), time (X3, 60–120) and TBAH concentration (X4, 50–60) (Table 1).

2.5. Dilute acid hydrolysis of hemicellulose

The dilute acid hydrolysis of hemicellulose was performed in a 250-ml flask. A mixture containing 10 g of the extracted hemicellulose and 100 mL of 7% sulfuric acid was heated at 100 °C for 2 h. The resultant hemicellulose hydrolysate was collected by filtration.

2.6. Detoxification of hemicellulose hydrolysate

Hemicellulosic hydrolysate was mixed with activated charcoal at 5% (w/v) and agitated at 200 rpm and 60 °C for 30 min. The mixture was filtered to remove charcoal. Untreated and charcoal-treated samples of hemicellulosic hydrolysate were neutralized by first adjusting to pH 10 with calcium carbonate at 70 °C for 10 min, then adding sodium sulfite to a final concentration of 0.1% and adjusting pH to 7.0 with phosphoric acid. Subsequently, the solutions were gathered by filtration to be used later.

2.7. Seed culture preparation

C. tropicalis CICC1779 cells from the slant were aseptically inoculated to 50 mL of medium for seed culture in 250 mL flasks and cultivated at 30 °C, 210 rpm for 38 h.

2.8. Xylitol production from hemicellulosic hydrolysate

Xylitol production was investigated in a 250-mL flask containing 50 mL of medium (pH 6.0) with 60 g of pure xylose or xylose in hemicellulosic hydrolysate supplemented with the aforementioned nutrients. The mixtures were inoculated with 10% (v/v) of seed culture and then cultivated at 30 °C. The shaker was operated at 210 rpm for initial 24 h and at 180 rpm for the following 24 h. Samples were taken periodically for analysis.

2.9. Analytical methods

FT-IR spectra of hemicellulose samples were recorded on FT-IR spectrophotometer (Nanometrics QS-1200) using a KBr disc in the range 4000–400 cm−1 with resolution of 4 cm−1. The analysis of xylose and xylitol was carried out on an Agilent 1260 Infinity HPLC system equipped with a refractive index detector and ZORBAX carbohydrate column (4.6 × 150 mm, 5 μm) using 75% acetonitrile (v/v) as the mobile phase at a flow rate of 2 mL/min and 30 °C.
Table 1
Experimental design and results of hemicellulose extraction by TBAH.

<table>
<thead>
<tr>
<th>Index</th>
<th>X1: Liquid/solid ratio (v/w)</th>
<th>X2: Temperature (°C)</th>
<th>X3: Time (min)</th>
<th>X4: TBAH concentration (%)</th>
<th>Response: Yield of hemicellulose extraction (%)</th>
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![Graph showing FT-IR spectra of corncobs and birchwood hemicelluloses.](image)

**Fig. 2.** FT-IR spectra of corncob and birchwood hemicelluloses.

3. Results and discussion

3.1. FT-IR spectra of hemicelluloses

As can be seen from Fig. 2, the FT-IR spectrum of the extracted corncobl hemicellulose is consistent with that of birchwood hemicellulose in the region of 400–4000 cm\(^{-1}\). The absorbance at 1380, 1168, 1050, and 900 cm\(^{-1}\) in the spectra are associated with hemicelluloses (Sun et al., 2005). The band at 1050 cm\(^{-1}\) is the typical specific absorbance of the C–O and C–C stretching and the glycosidic linkages for xylans (Ruiz et al., 2013). The broad absorption band at 3437 cm\(^{-1}\) is attributed to hydroxyl groups, and the band at 2928 cm\(^{-1}\) and 1380 cm\(^{-1}\) is credited to C–H stretching vibrations and bending vibration (Peng et al., 2009), and the sharp signal at 900 cm\(^{-1}\) is assigned to β-glycosidic linkages (1 → 4) between xylose units (Sun, Tomkinson, Wang, & Xiao, 2000).

3.2. Response surface optimization

Response surface methodology (RSM) is an efficient tool to optimize the parameters in experiments by establishing a relationship between variables and responses. Recently, the extraction
of celluloses and hemicelluloses have been optimized by RSM (Panthapulakkal & Sain, 2013; Wang, Song, Hou, Jia, & Yao, 2013). In this study, 29 full factorial designs based on the Box–Behnken design was used to optimize the factors affecting hemicellulose extraction by TBAH. The observed responses along with design matrix are indicated in Table 1. The following second-order regression equation illustrated the relationship of hemicellulose extraction by TBAH with respect to liquid/solid ratio, temperature, time, and TBAH concentration:

\[
y = 77.65 + 1.12X_1 + 1.43X_2 + 1.53X_3 + 0.11X_4 + 0.062X_1X_2 \\
- 0.21X_1X_3 - 0.46X_1X_4 - 0.43X_2X_3 - 0.17X_2X_4 + 0.12X_3X_4 \\
+ 0.91X_1^2 - 1.44X_2^2 - 1.66X_3^2 - 3.40X_4^2
\]

where \( y \) is the hemicellulose extraction yield and \( X_1, X_2, X_3, \) and \( X_4 \) are the liquid/solid ratio, temperature, time, and TBAH concentration, respectively.

The results were analyzed by ANOVA, as is shown in Table 2. The statistical significance of the models was evaluated by \( F \)-test analysis of variance, and values of \( P \) greater than 0.1 indicate that model terms are not significant. In the present study, the value of \( P \) less than 0.0001 implies that the model is highly significant. The coefficient of determination \( (R^2) \) of 0.9693 means it explains 96% of the variability in the responses for the region studied. The optimum conditions for maximizing the hemicellulose extraction calculated from the equation were as follows: time of 90.37 min, temperature of 59.96 °C, liquid/solid ratio of 12 (v/w), and TBAH concentration of 55.19%. The maximum hemicellulose extraction yield was calculated to be 79.66% according to the equation. We performed the batch experiments under optimized conditions listed as fol-

Fig. 3. Xylitol production from xylose and untreated corncob hemicellulosic hydrolysate by \textit{C. tropicalis}. (a) xylose; (b) untreated corncob hemicellulosic hydrolysate.
lows: time of 90 min, temperature of 60 °C, liquid/solid ratio of 12 (v/w), TBAH concentration of 55%. Results showed the hemicellulose extraction yield was 80.05% based on three repeated experiments, which was very close to the predicted response by the regression model.

### 3.3. Xylitol production from untreated corncob hemicellulosic hydrolysate

To investigate the feasibility of xylitol production from corncob hemicellulosic hydrolysate, we conducted fermentations by C. tropicalis using the untreated corncob hemicellulosic hydrolysate as a substrate and xylose as a control. As shown in Fig. 3, the xylitol yields for the untreated corncob hemicellulosic hydrolysate and xylose reach 0.4 and 0.84 g/L respectively. Previous studies have showed that xylose and glucose can be decomposed into furfural and hydroxymethylfurfural, respectively, and degraded further into formic acid and acetic acid at high temperatures (Olsson & Hahn-Hägerdal, 1996). Meanwhile, phenolic compounds may be generated from lignin degradation during this process (Parajó, Dominguez, & Dominguez, 1998). All of these chemicals are toxic to microorganisms and have a great impact on xylitol production. In the present study, it is clear that the possible inhibitors formed during hemicellulose extraction and acid hydrolysis would significantly affect xylitol production from untreated hemicellulosic hydrolysate.

### 3.4. Xylitol production from detoxified corncob hemicellulosic hydrolysate

Although several reports have shown that yeast can ferment non-detoxified hemicellulosic hydrolysate to xylitol, the detoxification of hemicellulosic hydrolysates is still required for xylitol production (Huang, Jiang, Guo, & Hwang, 2011; Ping, Ling, Song, & Ge, 2013). Many methods to detoxify hemicellulosic hydrolysates have been attempted (Jönsson, Alriksson, & Nilvebrant, 2013). With respect to the fermentability of the detoxified hemicellulosic hydrolysate, various methods used in hemicellulosic hydrolysate detoxifications may differ significantly (Cantarella, Cantarella, Gallifuoco, Spera, & Alfani, 2004). Among these methods, activated charcoal treatment and pH adjustment have been regarded as the most cost effective methods to remove toxic chemicals from
hydrolysates (Chandel, Kapoor, Singh, & Kuhad, 2007; Mussatto & Roberto, 2004).

The fermentation profile of xylitol production from corn cob hemicellulosic hydrolysate treated by activated charcoal, with the xylitol yield rising to 0.52 g/g and increasing by 30% compared to fermentation of the untreated hydrolysate was depicted in Fig. 4. Canilha, Carvalho, das Graças Almeida Felipe, and de Almeida e Silva (2008) reported similar results that a maximum xylitol yield of 0.54 g/g could be achieved by Candida guilliermondii FTI 20037 when fermenting wheat straw dilute sulfuric acid hydrolysate with activated charcoal treatment. de Albuquerque et al. (2015) evaluated xylitol production using cashew apple bagasse hydrolysate with different forms of activated charcoal pretreatment as a starting material for Kluyveromyces marxianus CCA510. This study showed that activated charcoal can reduce the amount of acids and phenolic compounds in hydrolysate and a maximum xylitol yield of 0.36 g/g was observed. Similarly, Parajó, Domínguez, and Domínguez (1995) found Debaryomyces hansenii NRRL Y-7426 can ferment wood hydrolysate with powdered activated charcoal treatment to xylitol in a yield of 0.32–0.35 g/g.

The results given in Fig. 5 showed xylitol production from corn cob hemicellulosic hydrolysate with pH adjustment. A maximum xylitol yield of 0.62 g/g can be observed after 36 h of fermentation. The xylitol yield was increased by 55% when the pH adjustment protocol was performed. A previous study showed C. tropicalis could ferment corn fiber dilute acid hydrolysate with pH adjustment to xylitol with a relatively low yield of 0.17 g/g (Buhner & Agblevor, 2004). Similar research carried out by Converti, Domínguez, Perego, da Silva, and Zilli (2000) gave a 0.46 g/g xylitol yield when Pachysolen tannophilus was employed to ferment wood hydrolysate with pH adjustment to xylitol. Significantly, the addition of sodium sulfite may be responsible for the increase of xylitol yield. A study showed that reducing reagents, e.g. sodium

**Fig. 5.** Xylitol production from detoxified corn cob hemicellulosic hydrolysate with pH adjustment by C. tropicalis.

**Fig. 6.** Xylitol production from corn cob hemicellulosic hydrolysate with pH adjustment and activated charcoal treatment combined by C. tropicalis.
sulfite, could improve the fermentation efficiency of hydrolysate (Alriksson, Cavka, & Jónsson, 2011).

Based on the above results, we combined pH adjustment and activated charcoal treatment to maximize detoxification of corncob hemicellulosic hydrolysate and xylitol yield. As is shown in Fig. 6, a maximum xylitol yield and productivity of 0.77 g/g and 2.45 g/(L.h), respectively, was attained using a combination of pH adjustment and activated charcoal treatment for corncob hemicellulosic hydrolysate. Xylitol yield can be largely increased by using corncob hemicellulosic hydrolysate with pH adjustment and activated charcoal treatment. The increase in xylitol yield and productivity should be contributed to removal of inhibitors through pH adjustment and activated charcoal treatment combined.

Substantial processes have also been evaluated to ferment hemicellulosic hydrolysate from various starting materials for xylitol production as listed in Table 3. Various combined detoxification methods have been used to improve the fermentability of hemicellulosic hydrolysates in these processes, and a comparison of results indicates that the xylitol yield varies from one method to another. As a method with good operability and low cost, pH adjustment and activated charcoal treatment combined have been used in many processes. For instance, Misra, Raghuvanshi, and Saxena (2013) reported that this protocol enabled C. tropicalis to ferment detoxified hydrolysate to xylitol with a xylitol yield of 0.5 g/g. When Eucalyptus hemicellulosic hydrolysate treated by activated charcoal combined with pH adjustment was used for xylitol production by Candida guilliermondii FTI20037, a xylitol yield of 0.4 g/g was achieved (Canilha, de Almeida e Silva, & Solenzal, 2004). Similar results reported by Buhner and Agblevor (2004) showed a maximum xylitol yield of 0.4 g/g can be obtained for fermentation of corn fiber hydrolysate with pH adjustment and activated charcoal treatment combined by C. tropicalis. Although these previous observations have showed hemicellulosic hydrolysate treated by pH adjustment and activated charcoal treatment combined produced a low conversion of xylose into xylitol. However, similar protocol was adopted to treat rice straw hemicellulosic hydrolysate for subsequent fermentation by C. guilliermondii FTI 20037 and produce a better xylitol yield peaking at 0.7 g/g (Mussatto & Roberto, 2001). In our study, we used this protocol to produce a maximum xylitol yield than all listed results above, therefore we conclude that fermenting corncob hemicellulosic hydrolysate with pH adjustment and activated charcoal treatment to xylitol by C. tropicalis is a favorable route.

Table 3 Comparison of xylitol production in different processes.

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<th>Feedstocks</th>
<th>Microorganisms</th>
<th>Methods of detoxification</th>
<th>Xylitol yield (g/g)</th>
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<td>Hardwood hydrolysate</td>
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</table>

4. Conclusions

In the present study, we have investigated a protocol to extract hemicellulose from corncob by using TBAH. Based on RSM, the conditions for corncob hemicellulose extraction by TBAH were optimized as follows: time of 90 min, temperature of 60 °C, liquid/solid ratio of 12 (v/w), and TBAH concentration of 55%. The corncob hemicellulose extraction performed under the optimized conditions resulted in a yield of 80.07%. The FT-IR spectrum of the extracted corncob hemicellulose is consistent with that of birchwood hemicellulose in the region of 400–4000 cm−1 with the specific absorbance at 1380, 1168, 1050, and 900 cm−1 being associated with hemicelluloses. After dilute acid hydrolysis of the extracted hemicellulose, the resulting corncob hemicellulosic hydrolysate was used in evaluating the feasibility of xylitol production by C. tropicalis. Finally, we found that combining pH adjustment and activated charcoal treatment of corncob hemicellulose hydrolysate greatly increased xylitol yield and productivity, to 0.77 g/g and 2.45 g/(L.h) respectively.

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References


