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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 55%, 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.

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 hydrolysate 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 (Ghindea, Csutak, 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.

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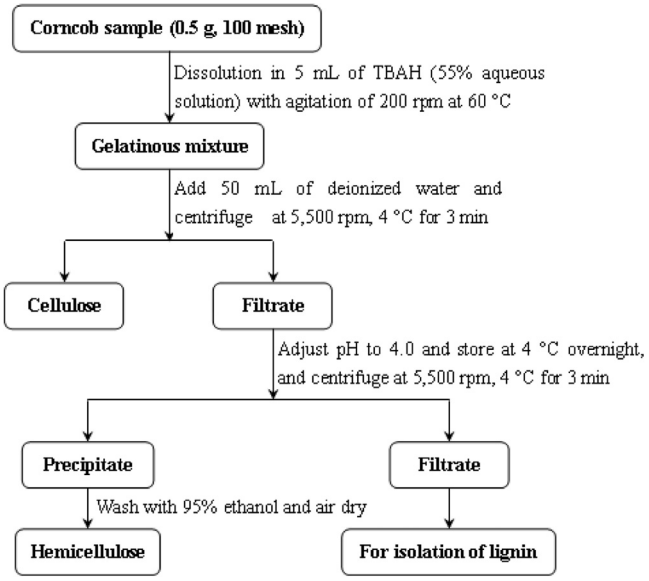

Fig. 1. Scheme for extraction of hemicellulose from corncob by TBAH.

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, xylose 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, $MgSO_4$ 0.2, KH_2PO_4 5. Xylitol production was performed in a medium containing xylose with the following composition (g/L): yeast extract 2.5, peptone 2.5, KH_2PO_4 5, $MgSO_4$ 0.5, $(NH_4)_2SO_4$ 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 hemicellulosic hydrolysate was collected by filtration.

2.6. Detoxification of hemicellulosic 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.