Xylitol Production from Prehydrolysis Liquor of Kraft-based Dissolving Pulp by *Candida tropicalis*

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Xylitol production from the hemicelluloses of prehydrolysis liquor (PHL) is of practical interest from both economic and environmental standpoints. The removal of the inhibitors, *e.g.*, lignin, acetic acid, and furfural, is the key to improving the conversion of xylose to xylitol when *Candida tropicalis* fermentation is used. For this purpose, a full chain process involving activated carbon adsorption, ion exchange resin treatment, acidolysis, and fermentation was considered in this work. The results showed that 72.6% of lignin and 67.1% of furfural were removed using an activated carbon dosage of 20 mg/g PHL. Additionally, 61.2% of acetic acid was also removed at a resin dosage of 100 mg/g PHL. Subsequently, acidolysis using sulfuric acid and pH adjustment with lime was performed on the treated PHL to convert the oligosaccharides into monosaccharides; a yield of 0.40 g xylitol/g xylose was achieved *via* treating the PHL with *Candida tropicalis* fermentation at 30 °C, 200 rpm, and 96 h. In addition, a material balance was determined for the full chain process.

Keywords: Xylitol; Prehydrolysis liquor; Fermentation; Activated carbon; Resin

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INTRODUCTION

Prehydrolysis kraft pulping has attracted appreciable interest in recent years for the production of dissolving pulps and hydrolysates (Wang *et al.* 2015a; Duan *et al.* 2017). It fits well into the biorefinery concept, as it effectively separates cellulose, hemicelluloses, and lignin from the biomass (Van Heiningen 2006; Sixta *et al.* 2013); the generated products may have different applications in biomass and bioenergy productions (Kaur and Ni 2015; Wang *et al.* 2015b).

In this process, hemicelluloses are mostly extracted from wood or other lignocellulosic materials in the prehydrolysis stage into the prehydrolysis liquor (PHL) (Borrega *et al.* 2013; Ludwig *et al.* 2013; Sharazi *et al.* 2017). The PHL contains chemicals with many potential uses, but it is currently underutilized; it is often mixed with black liquor and burned in the recovery boiler in the pulp mill for energy recovery (Fatehi *et al.* 2013; Liu *et al.* 2014). The co-existence of polysaccharides, monosaccharides, lignin, acetic acid, and furfural is the reason for the underutilization of PHL (Wang *et al.* 2014). It is well known that a part of the lignin is dissolved during prehydrolysis along with the hemicelluloses through the cleavage of aryl ether bonds (Yang *et al.* 2013a). Acetic acid is also released from the bound acetyl groups of these hemicelluloses, particularly when a

mixture of hardwood is used as the raw material (Li *et al.* 2014). Furfural, although found in relatively low concentrations, is also generated by the extensive degradation of pentoses by acid (Ahsan *et al.* 2013).

The separation and purification of the hemicelluloses and their degradation products is the key for the downstream utilization of PHL. Many technologies have been investigated for this purpose. For example, Shi *et al.* (2012) reported that the maximum lignin removal of 46% can be achieved when using polyethylene oxide (PEO) to treat PHL. Yasarla and Ramarao (2012) reported that polyethylene imine (PEI) and cationic polyacrylamides (cPAM) are also effective at lignin removal for PHL. Activated carbon is another promising approach for the removal lignin from PHL; a lignin removal of 80% at a charge of 3.3% activated carbon has been reported (Shen *et al.* 2013). Apart from inhibitor removal, the utilization of PHL has also been investigated. For example, Liu *et al.* (2013) studied furfural production from PHL through mono-/biphase systems and found that furfural yield reached as high as 69.8% (Liu *et al.* 2013). Yang *et al.* (2013b) reported that 65.13% of acetic acid can be recovered from PHL *via* reactive extraction with triisooctylamine, which also purified the sugars of PHL. However, there is still a lack of information regarding xylitol production from industrial PHL, particularly in a full chain design.

Xylitol is a naturally occurring pentahydroxy sugar alcohol that has a wide variety of food, pharmaceutical, and odontological applications (Manaf *et al.* 2018). It is commercially produced from lignocelluloses (*e.g.*, corncob) using an expensive Raney-nickel catalyst combined with harsh reaction conditions (*i.e.*, pressures of 10 to 15 atm and 130 °C); the process is costly and is not environmentally compatible (Zhang *et al.* 2014). Faced with the growing demand for xylitol and with the high amounts of underutilized PHL, the development of xylitol production from PHL *via* a bioconversion method is of practical interest. The bioconversion of xylose into xylitol using hydrolysates obtained from acid hydrolysis of rice straw has been reported by Mussatto *et al.* (2004). The improved xylitol yield from a model xylose solution was also observed by Zhang *et al.* (2018) when using xylitol dehydrogenase and alcohol dehydrogenase from *Gluconobacter thailandicus* (Zhang 2018).

The objective of this study was to develop a full chain process using commercial PHL as the feedstock to produce xylitol as the end product. Activated carbon and ion exchange resin were employed to remove the inhibitors (*e.g.*, lignin, furfural, and acetic acid) from the PHL. Acidolysis with sulfuric acid was employed to convert oligosaccharides into monosaccharides, which was the first step in the fermentation process with yeast (*Candida tropicalis*). In addition, a material balance of the full chain process was performed.

EXPERIMENTAL

Materials

Prehydrolysis liquor was collected from Shandong Sun Paper Co., Ltd. (Shandong, China), which produces dissolving pulp using prehydrolysis kraft technology; the pulp mill uses mixed hardwood as the raw material. Suspended solids and impurities contained in the raw PHL were filtered using Whatman qualitative filter paper according to a previously published procedure (Wang *et al.* 2014).

Commercial activated carbon was provided by Haiyan Active Carbon Co., Ltd. (Guangzhou, China). It is a wood-based powder and activated by equilibrating it with 0.5 M NaOH and 0.5 M HCl solution for 24 h, in succession, which was followed by washing with deionized water and oven-drying at 105 °C for 24 h.

Commercial ion exchange resin (A111S) was purchased from Huakai Resin Co., Ltd. (Shandong, China). It is a macroporous polystyrene resin grafted with tertiary amine groups.

The yeast, *Candida tropicalis*, was kindly supplied from the State Key Laboratory of Microbial Technology of Shandong University (Jinan, China), and was used for the bioconversion of xylose to xylitol. The yeast extract and peptone were all BioPure grade and purchased from AOBOX Biotechnology Co., Ltd. (Beijing, China). Xylitol was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Xylose and glucose were purchased from Sigma-Aldrich (Shanghai, China). The $MgSO_4$ and KH_2PO_4 were provided by Calvin Chemical Technology Co., Ltd. (Jinan, China).

Methods

Detoxification process

Activated carbon was added to a flask containing PHL. The mixture was shaken at 350 rpm, 20 °C, and for 2 h. The mixture was then filtered using a Whatman nylon membrane to collect the filtrated PHL (Wang *et al.* 2014). Ion exchange resin was added to the flask containing the treated PHL, and the mixture was shaken at 150 rpm, 20 °C, and for 5 h. After filtration, the ion-exchanged PHL was recovered; it was treated by acid hydrolysis under the conditions of 4% H_2SO_4, 121 °C, and 1 h in an autoclave. Afterwards, lime was used to adjust pH of the treated PHL to 5.5, which was compatible for the yeast fermentation treatment.

Yeast cultivation and fermentation

The yeast of *C. tropicalis* was maintained at 4 °C on malt extract agar slants. Cultivations were prepared by growing cells in 250-mL Erlenmeyer flasks; the medium (1 L) was composed of 30 g xylose, 10 g glucose, 10 g yeast extract, 2.5 g peptone, 0.2 g $MgSO_4$, and 5 g KH_2PO_4. Cultivations in 250-mL Erlenmeyer flasks were performed at 30 °C with the flasks being shaken with a rotatory shaker for 24 h. The cells were then added directly into the sterilised PHL (0.1 MPa for 15 min) for fermentation, which was conducted at 30 °C and 200 rpm in 250-mL Erlenmeyer flasks at a yeast charge of 10 g/L.

Analysis

The lignin content of PHL was analyzed by ultraviolet visible (UV-vis) spectrophotometry (Thermo Fisher Scientific, Madison, USA) at 205 nm in accordance with a previous study (Wang *et al.* 2014). The concentrations of saccharides and xylitol in the PHL were determined using an ion chromatography (ICS 5000, Thermo-Fisher, Waltham, MA, USA) unit with an electron capture detector (ECD) and CarboPA100 (3 mm × 150 mm) and CarboPacMA1 (4 mm × 250 mm) columns, using 250 mM and 500 mM sodium hydroxide (NaOH), respectively, as the eluent at the flow rate of 0.3 mL/min with a sample volume of 25 μL. An indirect analysis of oligosaccharides *via* acid hydrolysis of PHL at 121 °C for 1 h was used because ion chromatography can only detect monosaccharides. The degraded by-products, *e.g.*, acetic acid and furfural, were analyzed by high-performance liquid chromatography (HPLC; LC-20A, Shimadzu, Kyoto, Japan) equipped with a Waters C18 symmetry column (4.6 mm × 150 mm, 5 μm) and a UV-vis

detector (set at 210 nm wavelength). Runs were performed at 30 °C with 0.1% phosphoric acid (H_3PO_4) as the eluent at 0.5 mL/min. All samples were analyzed in duplicates. The inhibitor removal selectivity (*IRS*) was calculated based on Eq. 1,

$$IRS = \frac{C_{i,0} - C_{i,t}}{C_{s,0} - C_{s,t}} \times 100\% \quad (1)$$

where $C_{i,0}$ is the initial concentration (g/L) of inhibitors (*i.e.*, lignin, acetic acid, and furfural), $C_{i,t}$ is the concentration (g/L) of inhibitors after treatment, $Cs_{,0}$ is the initial concentration (g/L) of saccharides, and $C_{s,t}$ is the concentration (g/L) of saccharides after treatment.

RESULTS AND DISCUSSION

Lignin and Furfural Removal by Activated Carbon Adsorption

For the xylitol production from dissolved hemicelluloses in PHL, the detoxification of the PHL can be the key of bioconversion because toxic compounds can hamper the subsequent fermentation process (Travaini *et al.* 2013). Lignin is considered as a major inhibitor that is present in PHL due to its relatively high concentration (*i.e.*, 17.9 g/L). In contrast, furfural can approach a concentration of 1.0 g/L in the PHL and can act as a fermentation inhibitor (De Mancilha and Karim 2003). Activated carbon adsorption was first examined for the removal of these compounds at various amounts, as shown in Fig. 1.

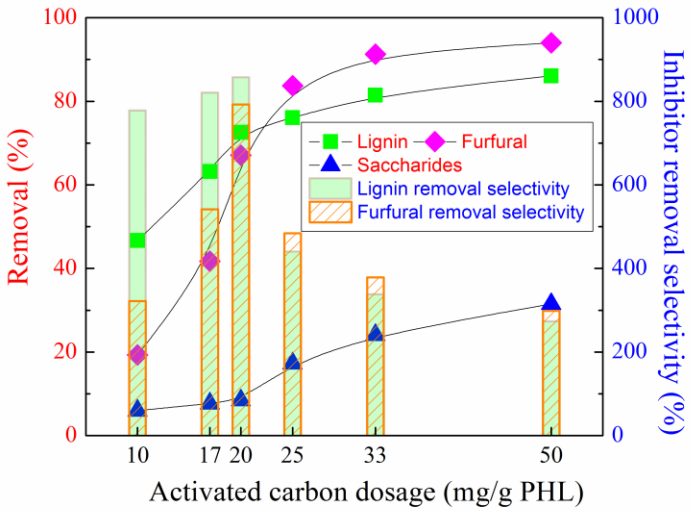

Fig. 1. The effect of activated carbon dosage on inhibitor removal from PHL; other conditions of inhibitor removal: 20 °C, 350 rpm, and 2 h

As can be seen in Fig. 1, the removal of lignin and furfural rapidly increased when the dosage increased from 10 to 25 mg/g PHL. Maximum removal levels of 86.1% and 94.0% for lignin and furfural, respectively, were obtained at an activated carbon dosage of 50 mg/g PHL. However, the loss of saccharides also increased with increasing activated carbon dosage, particularly at dosages higher than 25 mg/g. At least two hypotheses have been proposed to explain this phenomenon: (i) the affinity of saccharides to adsorb onto the activated carbon; and (ii) the existence of lignin-carbohydrate complexes and their

adsorption onto the activated carbon (Fatehi *et al.* 2016; Sharazi *et al.* 2017). Based on these results, the inhibitor removal selectivity of the system was also reduced at higher activated carbon dosages. An activated carbon dosage of 20 mg/g PHL resulted in lignin and furfural removal levels of 72.6% and 67.1%, respectively, while maintaining a 91.5% yield of saccharides in the PHL.

It should be noted that the activated carbon treatment was conducted at 3.6 pH (*i.e.*, the original pH of the PHL), which is supposed to adsorb more lignin than at higher pH levels as the acid groups of lignin are in their protonated forms at a low pH (Wang *et al.* 2015c). A lignin removal of 85% and a furfural removal of 65% was reported by Shen *et al.* (2013) when using activated carbon (PHL-to-activated carbon ratio of 30 g/g) to treat PHL at 3.6 pH; the authors noted an oligosaccharide and monosaccharide loss of approximately 25%. A furfural removal of 93% was observed by Lee *et al.* (2011) when using activated carbon at a 2.5 wt% to treat a synthetic PHL solution that was representative of PHL obtained from the steaming of mixed hardwood chips.

Acetic Acid Removal by Ion Exchange Resin Treatment

Acetic acid is another major fermentation inhibitor present in PHL at a concentration of 14.2 g/L. The effect of ion exchange resin on acetic acid removal is shown in Fig. 2. Acetic acid removal increased rapidly to 68.7% at a resin dosage of 10 to 100 mg/g PHL, and reached a plateau at a resin dosage of 200 mg/g PHL. Adsorption and ion exchange are the main mechanisms involved for acetic acid removal during ion exchange resin treatment (De Mancilha and Karim 2003). This treatment had limited sugar loss (< 10%). The optimized resin dosage was 100 mg/g PHL based on the removal selectivity evaluation. In one study, an acetic acid removal of 100% was achieved when studying the detoxification of hydrolyzate from the dilute acid hydrolysis of corn stover using the resin (type A103S) at a flow rate of 3.0 mL/min and total volume of 3.0 bed volume (De Mancilha and Karim 2003). In another study, the authors observed approximately 70% acetic acid removal when the PHL-to-resin ratio was 10 g/g, and they claimed that the pH shift from 4.0 to 6.0 when treated with resin was due to the weak basic nature of the resin (Shen *et al.* 2013).

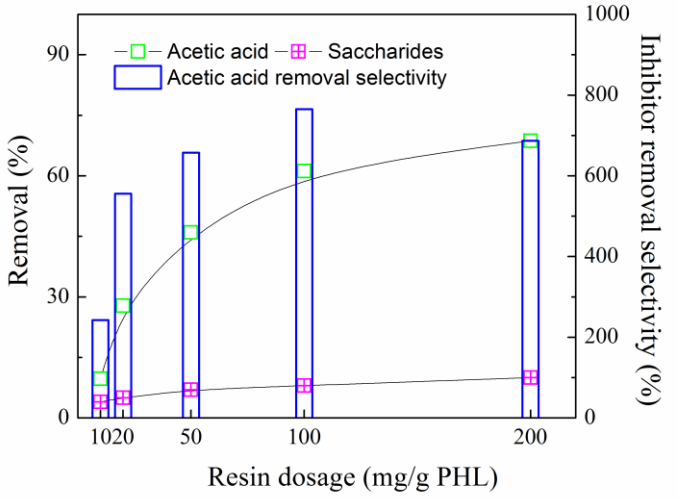

Fig. 2. Effect of ion exchange resin dosage on acetic acid removal from PHL; other conditions of inhibitor removal: 20 °C, 150 rpm, and 5 h

Fermentation Process of Treated PHL

Treatment of PHL by activated carbon and ion exchange resin facilitated the bioconversion of xylose when using *C. tropicalis* for fermentation. The results are presented in Fig. 3. Xylitol concentrations gradually increased with increasing fermentation time for the treated PHL after acidolysis, particularly at times greater than 24 h; xylitol concentration reached a plateau level at 72 h. The concentration of xylose decreased in accordance with the concentration increase of xylitol, albeit at a much faster rate, which implied an efficient conversion. However, the xylitol concentration was rather low (less than 5 g/L) for the treated PHL without acidolysis, which indicated that the monosaccharides were the predominant source for xylitol production. The fermentation of untreated PHL was also examined, and the observed xylitol concentration was zero (data not shown), which suggested that inhibiting substances blocked the bioconversion of xylose to xylitol. De Mancilha and Karim (2003) observed xylitol yields of 0.41 and 0.37 g/g xylose when using *Candida mogii* to bioconvert corn stover hydrolysate that had been treated by ion exchange resin. In another study, a xylitol yield of 31.8% was achieved when using *G. thailandicus* for the fermentation of a model xylose solution (Zhang *et al.* 2018).

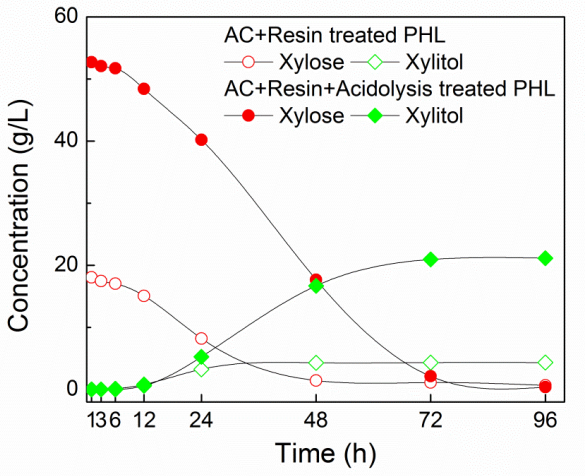

Fig. 3. Fermentation of AC and resin-treated PHL with and without acidolysis stage; other condition of fermentation: pH 5.5, 30 °C, 200 rpm, and 96 h

Material Balance

A material balance for the system is summarized in Fig. 4. The color of the raw PHL changed from dark to light due to the removal of lignin (72.6%) by adsorption onto the activated carbon. The acetic acid removal *via* activated carbon treatment was low (< 5%), which indicated that an ion exchange resin treatment was necessary. It was found that the losses of oligosaccharides and monosaccharides were negligible, particularly for xylose and xylan losses, which were less than 10%. Fortunately, most of the acetic acid (60.7%) was removed from the PHL by an ion exchange resin treatment. At this stage, the residual lignin and furfural levels were further reduced to an extremely low concentration (*i.e.*, 1.77 g/L and 0.08 g/L, respectively). Acid hydrolysis of PHL was performed to convert polysaccharides to monosaccharides, which caused the pH to decrease from 4.0 to 1.0. Lime was employed to adjust the pH to 5.5 prior to yeast fermentation, which resulted in a lignin removal of 2.6% and acetic acid removal of 23.2%. This facilitated downstream xylitol production. Finally, 21.2 g xylitol was produced at a yield of 0.40 g xylitol/g xylose.

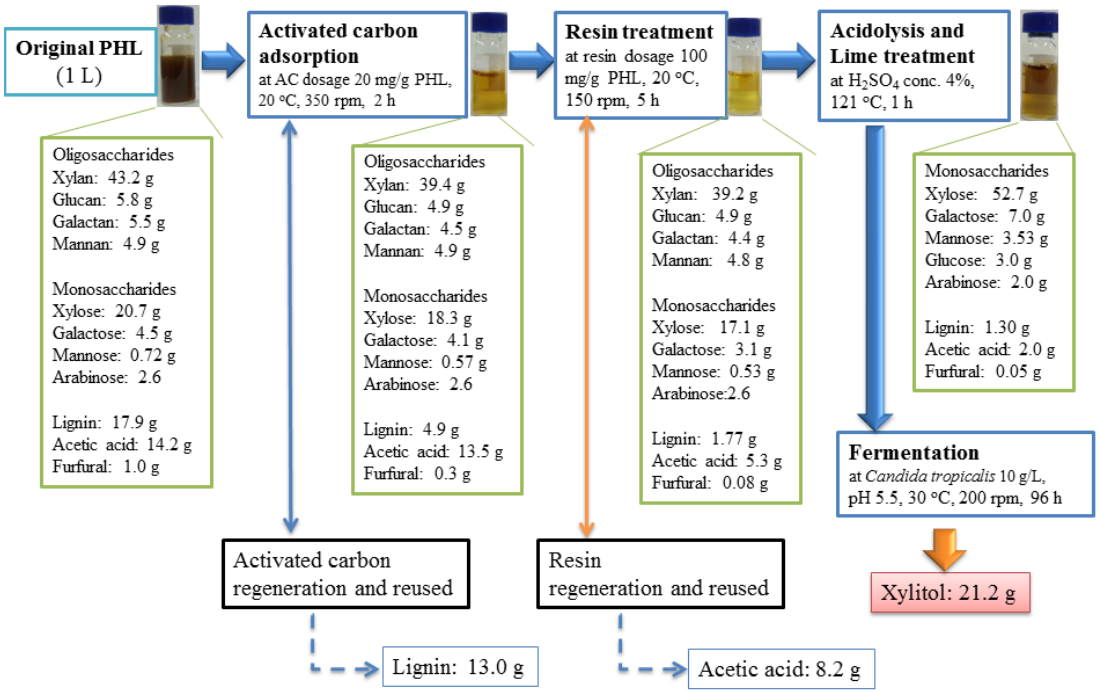

Fig. 4. Material balance of xylitol production under optimized condition

In this process, the activated carbon and ion exchange resin that were used can be regenerated and recycled. Thermal regeneration could be a practical process for regenerating spent activated carbon as reported by Gutsch and Sixta (2012) by using the recovered lignin as a fuel source. Organic solvent washing could also be used to regenerate activated carbon and recover the lignin for use in other applications, such as bioplastics, biofuels, and bio-phenols. In contrast, the used ion exchange resin could also be regenerated and reused by distillation, extraction, and sulfuric acid washing (Demiral and Yildirim 2003; Singh *et al.* 2006). Other than the regeneration and reactivation of the ion exchange resin, the acetic acid could also be recovered and considered as a value-added product. Mahnon (2010) desorbed acetic acid from ion exchange resins *via* sulfuric acid treatment and used it for polyethylene terephthalate resins and polyester fibers production.

CONCLUSIONS

1. A full chain process for xylitol production, involving sequential steps of activated carbon adsorption, ion exchange resin treatment, acidolysis, and fermentation, was investigated to convert xylose of PHL to xylitol.
2. Activated carbon removed most of the lignin and furfural from the PHL; subsequently, the ion exchange resin treatment removed most of the acetic acid. Acidolysis treatment converted the polysaccharides of PHL from oligomers into monomers, which facilitated the subsequent yeast fermentation. As a result, a yield of 0.40 g xylitol/g xylose was produced from the PHL.

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