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A Comparison of Low Temperature Alkali and High Temperature Acid Pretreatments for Improving Saccharification of Spent Mushroom Substrate

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Abstract

Low temperature alkali (LTA) pretreatment and high temperature acid (HTA) pretreatment methods were applied to spent mushroom substrate (SMS) for providing comparative performance data on the enhancement of enzymatic saccharification and the change of composition and structure. LTA pretreatment contributed to higher lignin removal (67.6%) and enzymatic digestibility (85.6%) during enzymatic hydrolysis, while HTA pretreatment resulted in higher hemicellulose reduction (85.3%) but lower enzymatic digestibility (43.5%). The physical structure was destroyed at certain degree and changes in cellulose crystallinity occurred after both LTA and HTA pretreatments. Besides, the effect of lignin reduction in alkali pretreatment on glucose yield was studied. These results indicated that LTA pretreatment had the potential to be developed into a cost effective process for producing bioproducts from lignocellulosic materials.

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Keywords: Spent mushroom substrate; Lignocellulose; Pretreatment; Reducing sugar; Delignification

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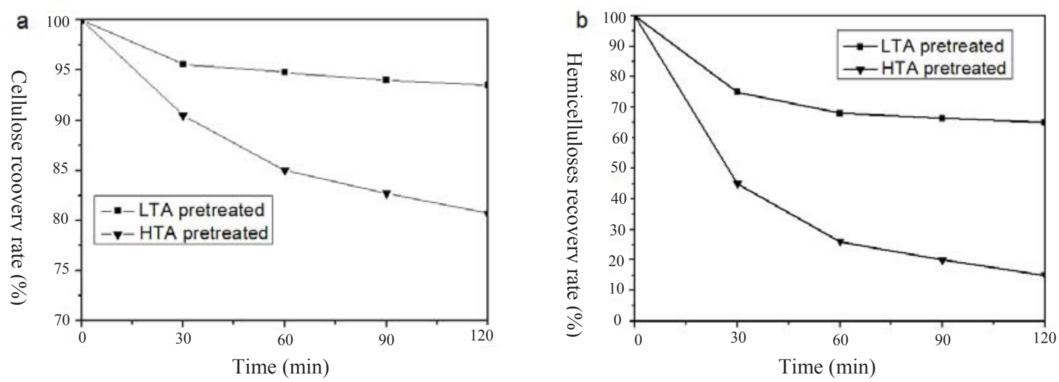
1. Introduction

Spent mushroom substrate (SMS), a byproduct of the mushroom industry is a kind of lignocellulosic materials. In China, more than 2 million tons of SMS were produced every year (Qiao et al., 2011), however, most of the SMS have been burnt for energy, which is neither environment-friendly nor economic. Furthermore, the lack of sustainable utilization strategies has become the limitative factor of the mushroom industry (Finney et al., 2009). SMS could be converted to reducing sugars for producing biofuels and other value-added bioproducts (Sun and Cheng, 2002). However, pretreatment by removing lignin and hemicellulose and breaking down the cellulose crystalline structure is required to overcome the problems caused by biomass recalcitrance for an effective cellulose conversion process. (Himmel et al., 2007). Sulfuric acid has been used to pretreat SMS (Qiao et al. 2011; Kapu et al. 2012) which involves high process temperature. Compared to acid pretreatment strategy, alkali pretreatment generally proceeds under lower temperatures and pressures (Sun and Cheng, 2002; Zhu et al., 2013). In this study, the effects of low temperature alkali (LTA) and high temperature acid (HTA) pretreatments on SMS were evaluated to provide comparative performance data.

2. Effects of pretreatments on SMS composition

Both LTA and HTA pretreatments obviously changed the SMS composition. Fig. 1a showed the recovery rates of cellulose and hemicellulose from SMS. Higher than 93% and 80% cellulose of raw SMS respectively was recovered after 120 min for LTA and HTA ptreatements which indicated that LTA pretreatment resulted in only minor cellulose loss. Fig. 1b presented the recovery rate of hemicellulose in LTA and HTA pretreated SMS. Compared with LTA pretreatment, HTA pretreatment removed more hemicellulose fraction from raw SMS. Only 26% of the initial hemicellulose remained in SMS after 60 min and the hemicellulose recovery rate decreased slowly with extended pretreatment to about 15% at 120 min. A remarkable reduction of lignin in SMS was observed at 30 min and the lignin recovery rate had no significant change as the time increased from 30 to 120 min in LTA pretreatment (Fig. 1c). In contrast, HTA pretreatment had minor impact on the lignin content of SMS. More detailed investigation was carried out to examine whether the removal of lignin had a big impact on improving enzymatic saccharification of pretreated SMS in Section 3.5.



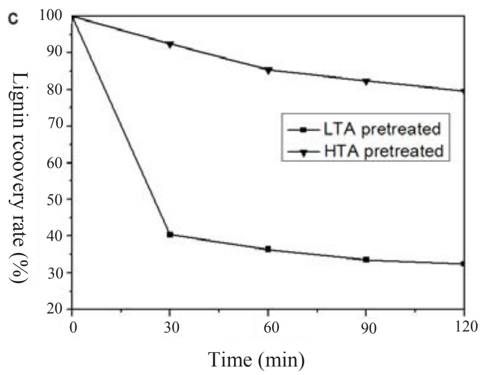


Fig. 1 Effects of LTA and HTA pretreatments on the cellulose (a), hemicellulose (b) and lignin (c) in SMS.

3. Effects of pretreatments on SMS structure

SEM images showed the untreated SMS sample presented a flat, smooth and order surface, representing a regular structure. In contrast, both the LTA and HTA pretreated samples showed a loose and disordered structure and missed some parts of the outer surface. The SEM images showed how the structure of native SMS changed after the pretreatment, which enlarged the contact area for enzymatic saccharification. Crystallinity was believed to be an important feature affecting enzymatic hydrolysis of lignocellulosic biomass. For pure cellulose, decreasing cellulose crystallinity could considerably improve the efficiency of enzymatic saccharification (Hall et al., 2010). However, when it comes to lignocellulosic biomass, the relations was more complicated due to its more complex components and structure. XRD profile of untreated, LTA pretreated and HTA pretreated SMS showed that two typical diffraction peaks were observed at $2\theta = 15.0°$ and $22.8°$ for all the samples, which corresponded to lattice planes (101) and (002). Compared to raw SMS, the peak (101) and peak (002) of LTA pretreated samples became weak and narrow, while HTA pretreated sample presented a similar peak (101) and broader peak (002). The CrI values were determined based on the XRD patterns. The CrI of untreated ample was 37.8% owing to the considerable amorphous components, especially hemicellulose and lignin in SMS. The CrI of LTA pretreated SMS was raised to 53.6%. The increase was mainly due to the removal of lignin which increased the relative amount of crystalline substances. The result suggested that during LTA pretreatment process under moderate conditions, there were little destruction to the crystal structure of cellulose. After HTA pretreatment, the CrI of SMS sample increased to 59.6%, which was probably caused by the significant removal of hemicellulose.

4. Effects of pretreatments on SMS enzymatic digestibility

Enzymatic saccharification of untreated, LTA pretreated and HTA pretreated SMS was performed with enzyme doses of 20 FPU cellulase per g of solids at 40 °C for 86 h. As shown in Fig. 2, the samples showed greatly different performance in enzymatic hydrolysis. Enzymatic digestibility was very low for untreated and HTA pretreated SMS with the maximum glucose yield of 30.6% and 43.5% respectively after 84 h of enzymatic hydrolysis. In contrast, LTA pretreatment significantly improved enzymatic saccharification of SMS which resulted in a maximum glucose yield of 84.6%. It was worthwhile to note that the rate of enzymatic hydrolysis before 48 h was very fast and reached a plateau at 36 h which indicated that shorter enzymatic hydrolysis time was acquired for LTA pretreated SMS.

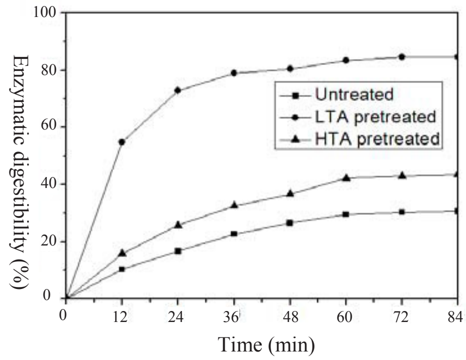


Fig. 2 Enzymatic hydrolysis of untreated, LTA pretreated and HTA pretreated SMS by incubating 20 FPU cellulase per g of solids at 40 °C for 86 h. Enzymatic digestibility was calculated from the maximum glucose yield.

5. Effects of lignin removal on SMS enzymatic digestibility

Lignin has been proved to greatly block the surface contact between cellulose and enzyme during the enzymatic saccharification process of lignocellulosic biomass. Removing the lignin content could improve the enzymatic digestibility efficiently (Yu et al., 2011). In this study, additional trials was performed to examine the relationship between lignin removal and enzymatic digestibility of LTA pretreated SMS. As shown in Fig. 3. An obverse correlation between the lignin removal and enzymatic digestibility with a correlation coefficient of 0.8765 was obtained which indicated that the elevation in lignin removal significantly enhanced the enzymatic saccharification of SMS. The result suggested that further improvement of delignification were critical to achieving ideal enzymatic digestibility.

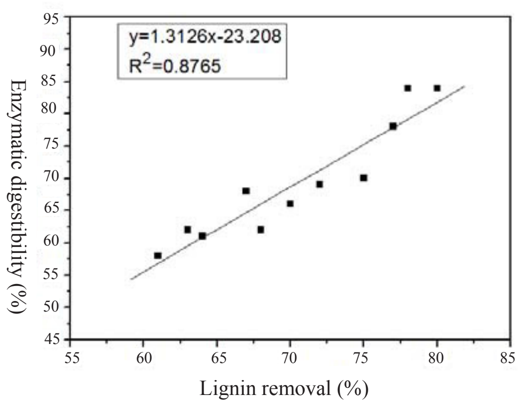


Fig. 3 Relationship between lignin removal and enzymatic digestibility of LTA pretreated SMS. Regardless of specific pretreatment conditions (alkali concentration, pretreatment time and temperature), 11 pretreated samples were selected.

6. Conclusions

LTA pretreatment mainly removing the lignin fraction was superior to HTA pretreatment in improving saccharification of SMS. There was a positive relationship between lignin removal and enzymatic digestibility in LTA pretreatment. Both LTA and HTA pretreatment could destroy the physical structure at certain degree and change the cellulose crystallinity. In conclusion, LTA pretreatment represented a cost effective method for achieving an efficient conversion of SMS to reducing sugars.

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