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Acid-Alkaline Two-stage Pretreatments of Corn Stover for Enhancing Enzymatic Saccharification

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Abstract. For the enzymatic saccharification of lignocellulosic biomass, single acid or alkaline pretreatment is not satisfactory because of the low sugar yields together with the neutralization of residual chemicals before enzymatic hydrolysis. Herein, an acid-alkaline two-stage pretreatment process was designed to treat corn stover. During the process, the pretreated liquid from the first stage and the solid residues from the second stage were mixed together for the subsequent simultaneous enzymatic hydrolysis, where a mixture of cellulase with an activity loading of 20 FPU/g substrate, cellobiase with an activity loading of 5 U/g substrate, and xylanase with an activity loading of 200 U/g substrate was used. Compared to the single acid or alkaline pretreatment, the acid-alkaline two-stage pretreatment could significantly improve the enzymatic saccharification, and 91.2% glucose yield with 52.56% of the theoretical total reducing sugar yield was achieved after the subsequent enzymatic hydrolysis.

Introduction

Recently, the conversion of biomass into liquid fuels has been paid more and more attention [1], due to the shortage of petroleum source. Corn stover, as an abundant agricultural waste, is a potential feedstock for ethanol production. However, in order to improve its enzymatic saccharification, a pretreatment step is needed because of the recalcitrant structure of lignocellulose [2].

Among the various chemical, mechanical, thermo-chemical, and biochemical pretreatment methods, dilute acid pretreatment and alkaline pretreatment are the two most extensively studied methods [3-6]. Hemicellulose can be efficiently removed during dilute acid pretreatment, but the delignification is relatively low. Moreover, the high temperature or high concentrations of acid catalyst can cause degradation of polysaccharide, forming some by-products that are often inhibitory to downstream fermentation [7].

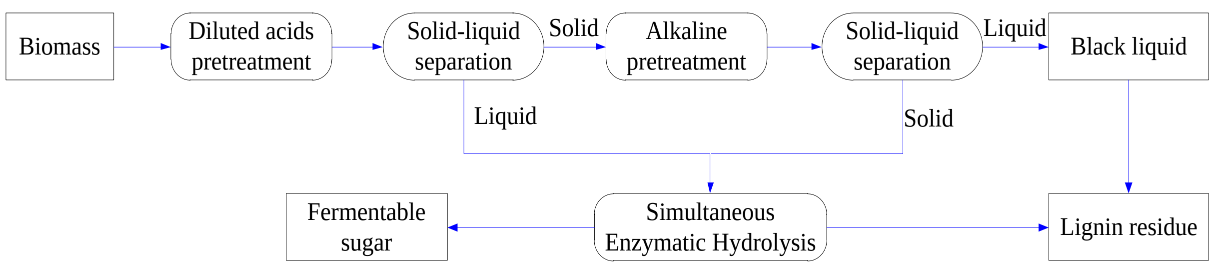

Fig.1. The overall process of the acid-alkaline two-stage pretreatment.

Alkaline pretreatment can lead to efficient delignification, the decrease of cellulose crystallinity, and the swelling of cellulose, thus enhancing the accessibility of enzymes to biomass [8]. However, it also can result in xylan losses. Furthermore, monomeric lignin compounds released by lignin degradation during alkaline pretreatment are potential inhibitors as well [7]. Thus, single acid or alkaline pretreatment is not satisfactory because of the low sugar yields as well as the neutralization of residual chemicals before enzymatic hydrolysis.

In this study, a two-stage pretreatment process (the dilute acid followed by alkaline pretreatment) was designed. The pretreated liquid from the acid stage and the solid residues from the alkaline stage were mixed together for the subsequent enzymatic hydrolysis, as shown in Fig. 1. The enzymatic saccharification of the two-stage pretreatment was investigated.

Experimental

Materials

The corn stover used was collected from Qingdao (Shandong province, China), and was cut to 2-3 cm length before pretreatment. The raw material (on the oven dry weight basis) consisted of 35.38% glucan, 24.47% xylan, 21.20% Klason lignin, as well as minor amounts of arabinan and galactan. The total reducing sugar of raw corn stover is 64.89%.

Commercial enzymes, Celluclast 1.5 L (cellulase), Novozyme 188 (β-glucosidase), and xylanase were purchased from Sigma-Aldrich. Sulfuric acid, sodium hydroxide, and other chemicals were used as received.

Pretreatment of corn stover

The overall process of the acid-alkaline two-stage pretreatment process was presented in Fig. 1. In the first stage of pretreatment, the corn stover was pretreated with 1% (w/w) sulfuric acid (solid to liquid ratio of 1:9) in an autoclave (Model 4566, Parr) at 120 ºC for 1 h. After the dilute acid pretreatment, the liquid and solid was separated, and then the collected solid residue was treated by 10 wt.% NaOH with the solid concentration of 12.5% at 145 ºC for 0.5 h. Subsequently, the pretreated liquid collected from the first stage and the solid recovered from the second stage (alkaline pretreatment) were mixed together for the simultaneous enzymatic hydrolysis to release fermentable sugar [9]. Alkaline lignin dissolved out in the second stage can be purified by acid-precipitation. The purified lignin can be used to produce surfactant or other chemicals [10].

For comparison of the pretreatment effect on enzymatic saccharification, the individual dilute acid and alkaline pretreatment was conducted, respectively, under the same conditions as described above.

Enzymatic saccharification

The enzymatic saccharification for different pretreated biomass was performed, respectively, following the standard procedure of National Renewable Energy Laboratory (NREL) [11].The mixture of cellulase with an activity loading of 20 FPU/g substrate, cellobiase with an activity loading of 5 U/g substrate, and xylanase with an activity loading of 200 U/g substrate was used for enzymatic saccharification.

Analysis methods

The moisture content was determined by drying the sample at 105 ^{o}C to a constant weight. The samples were then heated to 550 ^{o}C for 5 h to determine ash content. The monosaccharide of glucose and xylose were measured by DIONEX ICS-3000 using CarbopaPA10 column equipped. The 3, 5-dinitrosalicylic acid (DNS) assay method was used to detect total reducing sugars. All experiments and analysis were performed in triplicate under the same conditions, and the average value was reported.

Results and Discussion

Solid yield and glucan content of different pretreatment

Fig. 2(a) presented the solid yield (oven dry basis) of different pretreatment for corn stover. As can be seen from Fig. 2(a), the solid yield (about 55%) after dilute acid pretreatment under mild conditions (120 ^{o}C, 1% (w/w) sulfuric acid) was lower than that (about 58%) after alkaline pretreatment at 145 ^{o}C with NaOH dosage of 10%. This was because part of lignin was dissolved and most of hemicellulose was removed from the network structure of fiber cells during dilute acid pretreatment [12], while the majority of dissolved components during alkaline pretreatment were lignin. For the acid-alkaline two-stage pretreatment, most of hemicellulose was dissolved in the first stage of pretreatment, leading to the increase of porosity and specific surface area of the substrates, which facilitated the penetration, adsorption and reaction of alkali during the second stage of pretreatment. This could lead to more lignin removal at lower temperature with less chemical dose. Therefore, the solid yield after the two-stage pretreatment process was the lowest (42.7%) compared to the single acid or alkaline pretreatment.

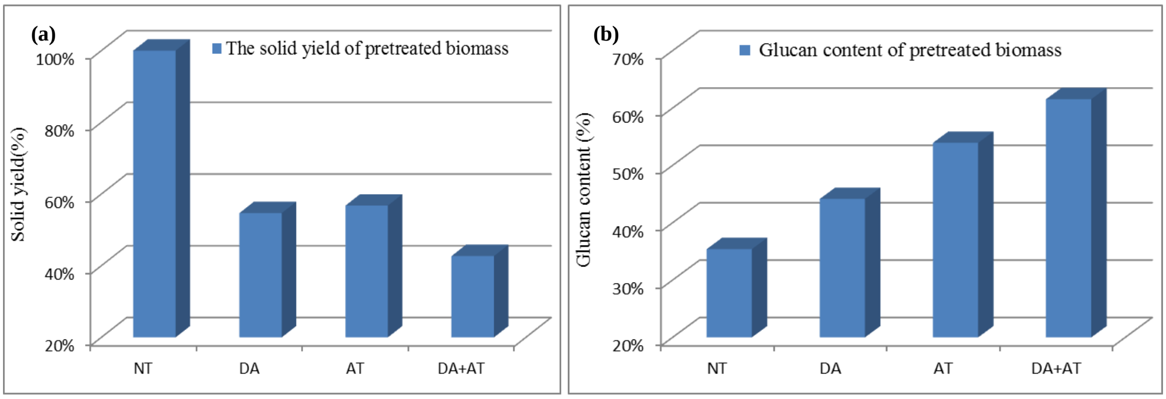

Fig.2. Solid yield (a) and glucan content (b) of different pretreatment for corn stover.
NT: non pretreatment; DA: dilute acid pretreatment; AT: alkaline pretreatment; DA+AT: acid-alkaline two-stage pretreatment

The glucan content of pretreated corn stover after different pretreatment methods was shown in Fig. 2(b). It was seen that, the glucan content of untreated corn stover was 35.38%, while the glucan contents of dilute acid pretreated and alkaline pretreated corn stover were 44.14% and 53.92%, respectively. As exhibited in Fig. 2(a), the solid yield after the dilute acid pretreatment was lower than that after the alkaline pretreatment, and the glucan content of acid pretreated corn stover was also lower than that of alkaline pretreated corn stover. This was probably due to the fact that a portion of cellulose was dissolved with the dissolving of hemicellulose during acid pretreatment [2], while alkaline pretreatment facilitated the lignin removal and some hemicellulose could be dissolved as well. Fig. 2(b) also presented that the highest glucan content of 61.53% was achieved after the acid-alkaline two-stage pretreatment process. This was because the acid pretreatment stage not only could remove most of hemicellulose, but also facilitate lignin removal in the subsequent alkaline pretreatment.

Enzymatic efficiency of different pretreatments

Enzymatic efficiency was estimated by the glucose yield of enzymatic hydrolysis for different pretreatments, which was displayed in Fig. 3. As can be seen, after enzymatic hydrolysis of pretreated substrate from the acid-alkaline two-stage pretreatment of corn stover, 92.3% of the theoretical glucose yield was obtained, which was significantly higher than that (45.65%) from the dilute acid pretreatment and the one (78.3%) from the alkaline pretreatment. Those results were consistent with the corresponding glucan content of pretreated corn stover after different pretreatments, as shown in Fig. 2 (b). In addition, when the pretreated liquid from the first stage was mixed with the pretreated substrate from the second stage for simultaneous enzymatic hydrolysis, the glucose yield of 91.2% was achieved, which was just slightly lower than that of enzymatic hydrolysis of the pretreated substrate from the second stage without the mixture of pretreated liquid from the first stage. Therefore, the simultaneous enzymatic hydrolysis after the acid-alkaline two-stage pretreatment just has minor impact on the glucose yield, in comparison with the separate enzymatic hydrolysis.

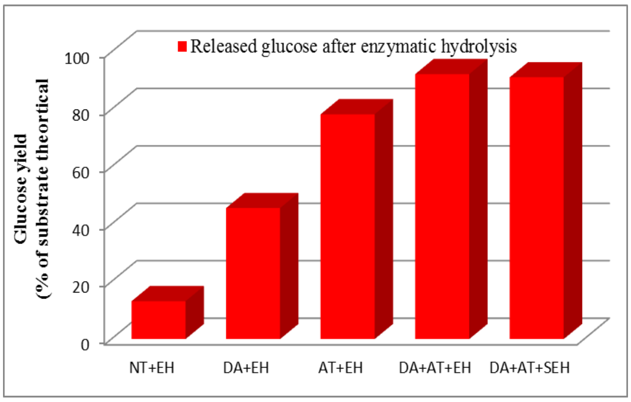

Fig.3. Glucose yield of enzymatic hydrolysis for different pretreatments

NT: non pretreatment; DA: dilute acid pretreatment; AT: alkaline pretreatment; DA+AT: acid-alkaline two-stage pretreatment; EH: enzymatic hydrolysis; SEH: simultaneous enzymatic hydrolysis

The yield of total reducing sugar after pretreatment and enzymatic hydrolysis

As shown in Fig. 4, which was the yield of total reducing sugar after pretreatment and enzymatic hydrolysis, 52.56% of the theoretical reducing sugar yield after the simultaneous enzymatic hydrolysis was obtained, which was slightly higher than the summation (52.18%) of the reducing sugar obtained from the pretreated liquid in the first stage and the reducing sugar from the enzymatic hydrolysis of the pretreated substrate in the second stage. However, most importantly, the total reducing sugar yield of the simultaneous enzymatic hydrolysis for the two-stage pretreatment was significantly higher than that of the single dilute acid or alkaline pretreatment, as presented in Fig. 4.

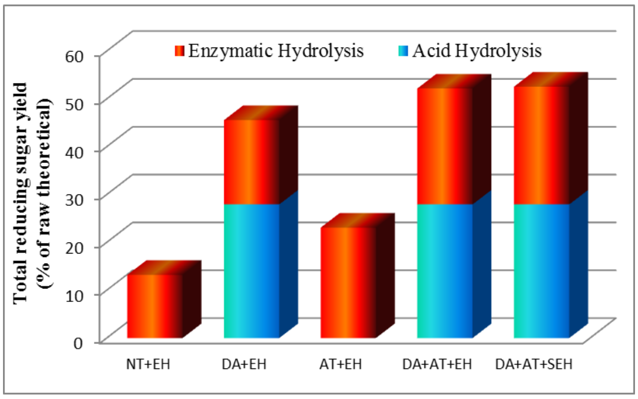

Fig.4. The yield of total reducing sugar after pretreatment and encymatic hydrolysis.

NT: non pretreatment; DA: dilute acid pretreatment; AT: alkaline pretreatment; DA+AT: acid-alkaline two-stage pretreatment; EH: enzymatic hydrolysis; SEH: simultaneous enzymatic hydrolysis

Conclusions

In this study, a two-stage (first dilute acid pretreatment and followed by alkaline pretreatment) process was designed to treat corn stover, and the effect of the two-stage pretreatment on the enzymatic saccharification was investigated in the comparison with the single dilute acid pretreatment as well as the single alkaline pretreatment. It was found that the two-stage pretreatment could significantly enhance the enzymatic saccharification, and 91.2% of glucose yield was obtained, which was much higher than that through the single acid pretreatment or alkaline pretreatment under the same corresponding conditions, due to the fact that the dilute acid pretreatment could facilitate both the removal of hemicellulose in the first stage and the delignification in the second stage. Furthermore, the simultaneous enzymatic hydrolysis of the mixture of the pretreated liquor from the first stage and the pretreated substrate from the second stage could not only reach the neutralization purpose, but also reduce the losses of hemicellulose, thus improving the enzymatic saccharification of the overall pretreatment process. Hence, the designed two-stage pretreatment process has large potential to achieve the commercial conversion of lignocellulosic biomass into fermentable sugars.

Acknowledgements

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