



Short Communication

Dry dilute acid pretreatment by co-currently feeding of corn stover feedstock and dilute acid solution without impregnation



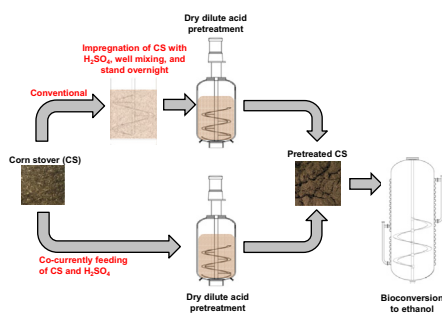
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HIGHLIGHTS

- Dry dilute acid pretreatment (DDAP) with co-currently feedstock feeding was performed.
- DDAP carried out in the reactor with helical agitator to improve mass transfer.
- Similar efficiency of DDAP obtained using impregnated and non-impregnated corn stover.

GRAPHICAL ABSTRACT



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ABSTRACT

Impregnation of lignocellulose materials with dilute acid solution is a routine operation in conventional dilute acid pretreatment. The dry dilute acid pretreatment (DDAP) at high solids content up to 70% is naturally considered to require longer impregnation time. In this study, a co-currently feeding operation of corn stover and dilute sulfuric acid solution without any impregnation was tested for DDAP. The DDAP pretreated corn stover without impregnation is found to be essentially no difference in pretreatment efficiency compared to those with impregnation in the helically agitated reactor. The yield from cellulose to ethanol in SSF again shows no obvious difference between the DDAP pretreated corn stover with and without impregnation. This study suggests that impregnation in DDAP was not necessary under the helical agitation mixing. The results provided a useful way of cost reduction and process simplification in pretreatment.

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1. Introduction

Impregnation of aqueous solutions with lignocellulose materials before pretreatment was a routine procedure for enhancing the pretreatment efficiency (Galbe and Zacchi, 2012). In the pulp and paper industries, impregnation is widely applied for enhancing the conversion from woody biomass to pulp (Viamajala et al., 2006). In cellulosic ethanol production process, impregnation of lignocellulosic materials using various solutions such as dilute acid solution (Sassner et al., 2008; Sørensen et al., 2008), alkali solution

(Kumar et al., 2011), hydrogen peroxide (Shao et al., 2013), aqueous ammonia (Murnen et al., 2007), or SO₂ gas (Rudolf et al., 2008) is also a routine operation in pretreatment step, then the impregnated materials are sent for pretreatment. It has been found that long time impregnation helps water or chemical substances penetrate into the interior regions or channels of lignocellulose biomass. The penetrated water or chemicals facilitates the partial hydrolysis of cellulose and hemicellulose into oligomers even at low temperature. As the outcome, the mixing and mass transfer of the pretreatment are enhanced and the pretreatment efficiency is improved.

In the conventional dilute acid pretreatment method, generally lignocellulose materials were completely impregnated into large

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bulk dilute acid solution at the high ratio of liquid to solid up to 10:1, then the whole slurry was pretreated under high temperature (160–220 °C) (Lloyd and Wyman, 2005). Recently a “dry dilute acid pretreatment” (DDAP) method was developed by significantly decreasing the usage of dilute acid solution in order to overcome the drawback of massive waste water generation in the conventional dilute acid pretreatment (Zhang et al., 2011). In the DDAP process, the solids portion (dry lignocellulose materials) is two folds greater than the liquid portion (aqueous dilute sulfuric acid solution). Thus both the feedstock and product are essentially solid materials without free water existence. Due to the high absorption capacity of lignocellulose materials to aqueous solution, the small amount of dilute acid solution is quickly absorbed by large lignocellulose bulk body without sufficient mixing and mass transfer. Therefore, lignocellulose materials impregnated by dilute acid solution are generally mixed thoroughly and then placed overnight (12 h) to let the dilute acid fully penetrate into the whole lignocellulose (Zhang et al., 2011; He et al., 2014). However, impregnation step creates troubles in the industrial scale operation. Additional transportation system, mixing equipment, and storage tanks are required which lead to the increase of capital investment. It is an ideal way to realize the dry pretreatment process if the impregnation step is complete cut from the process flowsheet.

In this study, an interesting test was carried out to check the possibility of impregnation removal. The dry corn stover materials and dilute acid solution were co-currently fed into the helically agitated reactor without impregnation, instead of the impregnation for hours. Surprisingly, the results show that the pretreatment efficiency by co-currently feeding without impregnation was essentially the same compared to that after long time impregnation. These results provided an important evidence for deleting the impregnation step in the DDAP process in large scale reactors, and could lead to a significant cost reduction and process simplification in the commercial production of cellulosic ethanol.

2. Methods

2.1. Raw materials and reagents

The virgin corn stover (CS) was grown in Henan, China and harvested in fall 2011. CS was washed and dried at 105 °C until the weight was constant, then was milled coarsely using a beater pulverizer and screened through a mesh with the circle diameter of 10 mm then stored in sealed plastic bags until use.

The cellulase enzyme Youtell #6 was kindly provided by Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The filter paper activity of Youtell #6 was 135 FPU/g determined by the NREL Laboratory Analytical Procedure (LAP) LAP-006 (Adney and Baker, 1996), and the cellobiase activity of 344 cellobiase units (CBU)/g determined by the method of Sharma et al. (1991).

Amorphotheca resiniae ZN1 was stored at Chinese General Microorganisms Collection Center, Beijing, China with the registration number of CGMCC 7452. The biotransformation for the removal of inhibitors from the pretreated corn stover was described in Zhang et al. (2010b) and He et al. (2014). *Saccharomyces cerevisiae* DQ1 was stored at Chinese General Microorganisms Collection Center, Beijing, China with the registration number of CGMCC 2528 (Zhang et al., 2010a; Chu et al., 2012). *S. cerevisiae* DQ1 was first adapted in the undetoxified hydrolysate of pretreated corn stover for 3 times and then inoculated into the bioreactors to start the fermentation.

2.2. Pretreatment reactor and operation

Pretreatment reactor was a stainless cylinder with a work volume of 20 L (260 mm in diameter and 400 mm in height) as

described in detail by He et al. (2014). The helical stirrer was driven and rotated by a motor mounted on top of the reactor through an electromagnetic convertor. The steam vapor was injected into the reactor from the bottom. Pretreatment operation was carried out at 185 °C for 3 min using 2.5 g of sulfuric acid per 100 g of dry corn stover (2.5% acid usage). The agitation rate was set to 50 rpm, and the control experiment of non-agitated condition was set to 0 rpm. Two pretreatment cases were tested using the impregnated corn stover and the co-currently feeding of corn stover with dilute acid solution.

The impregnation of corn stover by dilute acid solution was carried out by mixing the 1400 g of dry corn stover with 700 g of 5% (w/w) sulfuric acid solution thoroughly and then maintaining at ambient temperature (18–25 °C) in sealed plastic bags for 0.5, 12, and 24 h, respectively. Then the impregnated corn stover materials were fed into the pretreatment reactor and ready for pretreatment.

The co-currently feeding operation was carried out by feeding the dry corn stover materials and the dilute sulfuric acid solution into the reactor simultaneously under the helical agitation of 50 rpm. No impregnation of corn stover with dilute acid solution was done.

The pretreated corn stover was assayed by enzymatic hydrolysis following the NREL LAP-009 (Brown and Torget, 1996). One gram of the pretreated corn stover (dry base) was added into 10 ml of deionized water and then adjusted to pH 4.8 by 5 M NaOH solutions in 100 ml flask followed by adding 0.1 M citrate buffer (pH 4.8) to prepare a 5% (w/w) solids slurry in the flask. The cellulase dosage was 15 FPU/g DM (dry pretreated CS) and the hydrolysis lasted for 72 h at 50 °C and 150 rpm of shaking.

2.3. Simultaneous saccharification and ethanol fermentation (SSF)

Before SSF process was carried out, the pretreated corn stover was detoxified biologically by *A. resiniae* ZN1 in solid state culture to remove the inhibitors in the corn stover. During the biotransformation step, the fungus would consume the inhibitors such as furans and organic acid but less glucose and none of cellulose were degraded as described in Zhang et al. (2010b). The content of cellulose and hemicellulose in the detoxified CS was almost same with that in the non-detoxified CS. The detoxification step was stopped when the main inhibitors were removed. The SSF operation was carried out in a 5 L helical ribbon stirrer agitated bioreactor as described in Zhang et al. (2010a) with the detoxified CS as feedstock. The SSF operation was conducted at 25% solids (dry pretreated corn stover) concentration (w/w), 15 FPU/g DM of cellulase dosage. The operation started with 12 h prehydrolysis at 50 °C and pH 4.8, then the temperature was reduced to 37 °C and the adapted *S. cerevisiae* DQ1 cells were inoculated into the bioreactor at 10% inoculum ratio (v/v) to start the SSF. Samples were taken periodically for analysis of ethanol and glucose.

2.4. Measurement of cellulose and xylan

The cellulose and xylan content of corn stover were measured by two-step acid hydrolysis according to NREL LAPs (Sluiter et al., 2008a,b). One hundred milligram of dried corn stover was added to 1 ml 72% (w/w) H₂SO₄ for 60 min reaction and then hydrolyzed in 4% sulfuric acid solution at 121 °C for 60 min. The hydrolysate was taken for sugars determination to calculate the cellulose and xylan content.

Oligomers of cellulose and xylan were measured according to NREL LAP (Sluiter et al., 2008b). The mixture of 5 g wet pretreated corn stover and 50 ml deionized water was shook at 180 rpm for 2 h. 5 ml Supernatant was taken and reacted in 4% sulfuric acid concentration in the autoclave at 121 °C for 1 h. Then the hydrolysate was used for sugars determination. The difference of the sugar

concentration before and after acid hydrolysis was defined as the oligosaccharides content.

2.5. Sugar and inhibitor analysis

Sugars and inhibitors were measured by high-performance liquid chromatography (LC-20AD, RID-10A refractive index detector, Shimadzu, Kyoto, Japan) equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at the column temperature 65 °C. The mobile phase was 5 mM H₂SO₄ at the rate of 0.6 ml/min. All samples were centrifuged to remove the cell mass and other water insoluble substances, and then filtered through a 0.22 μm filter before analysis.

3. Results and discussion

3.1. Impact of impregnation on the pretreatment under helically agitation

The impact of impregnation of corn stover with dilute sulfuric acid solution on pretreatment efficiency was experimentally examined in the helically agitated reactor at high solids content up to 2:1 of dry solids to liquid. Fig. 1(a) shows that the cellulose conversion ratio of the pretreated corn stover to glucose in the enzymatic hydrolysis reached 84.77% when the dry corn stover and the dilute acid solution were co-currently fed into the reactor without any impregnation operation. As the comparison, the cellulose conversions ratios were 82.00%, 87.11%, and 84.43% when the corn stover was impregnated with the dilute acid solution for 0.5, 12, and 24 h, respectively. This result indicates that the cellulose conversion of the pretreated corn stover was essentially the same in the cases of non-impregnated (co-currently feeding of corn stover with dilute acid solution) and impregnated operations. Fig. 1(a) also indicates that the cellulose conversion at different impregnation time from 0.5 to 24 h did not give the obvious changes and the same cellulose conversion results were obtained.

For the further evaluation of the impact of impregnation, the pretreated corn stover obtained at different impregnation conditions were used for simultaneous saccharification and ethanol fermentation (SSF) at 25% solids loading. Fig. 1(a) shows that the ethanol yield after 72 h SSF was 79.80% when there was no impregnation operation (co-currently feeding of corn stover and dilute acid solution), comparing to 73.87%, 72.88%, and 77.92% with 0.5, 12, and 24 h impregnation, respectively. Again, the ethanol yield showed no essential differences for the pretreated corn stover with and without impregnation.

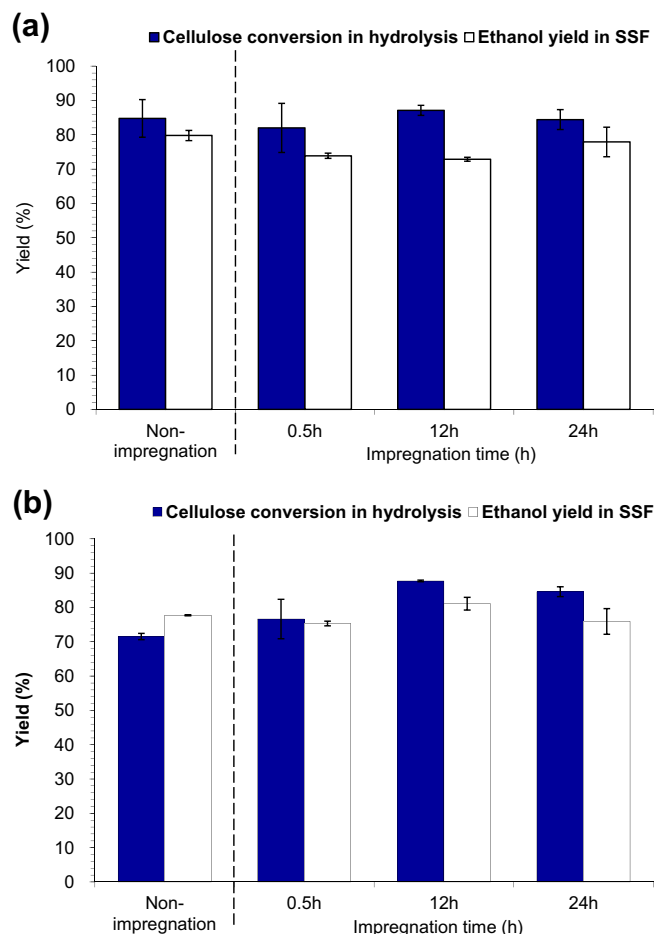


Fig. 1. Evaluation of dry dilute acid pretreatment (DDAP) under the different impregnation conditions. (a) In the helically agitated reactor; (b) in the non-agitated reactor. Hydrolysis conditions: 5% solids loading, cellulase 15 FPU/g DM, 50 °C, pH 4.8, 150 rpm for 72 h. SSF conditions: 25% solids loading, cellulase 15 FPU/g DM, 150 rpm, 10% (v/v) inoculum ratio for 72 h; 50 °C and pH 4.8 at prehydrolysis step; 37 °C, pH 5.5 at SSF step.

Tables 1 and 2 show that the composition of the corn stover materials before and after pretreatment at different impregnation conditions. For the virgin corn stover before the pretreatment, the insoluble components were approximately the same with and without impregnation (Table 1); cellulose increased slightly

Table 1

Composition of the insoluble components of corn stover during the dry dilute acid pretreatment (DDAP).

| | Cellulose content (%) | Xylan content (%) | Cellulose conversion ^a (%) | Ethanol yield ^b (%) |
|------------------------------|-----------------------|-------------------|---------------------------------------|--------------------------------|
| Original corn stover | 37.15 ± 0.22 | 19.86 ± 0.56 | | |
| CS before pretreatment | | | | |
| Without impregnation | 37.46 ± 0.53 | 20.92 ± 0.15 | \ | \ |
| Impregnation for 0.5 h | 37.67 ± 1.32 | 20.14 ± 0.73 | \ | \ |
| Impregnation for 12 h | 37.71 ± 0.81 | 20.88 ± 0.40 | \ | \ |
| Impregnation for 24 h | 38.38 ± 0.88 | 20.84 ± 0.31 | \ | \ |
| CS pretreated with agitation | | | | |
| Co-currently feeding mode | 39.02 ± 0.95 | 2.66 ± 0.05 | 84.77 ± 5.49 | 79.80 ± 1.49 |
| Impregnation for 0.5 h | 40.17 ± 2.04 | 2.33 ± 0.13 | 82.00 ± 7.15 | 73.87 ± 0.76 |
| Impregnation for 12 h | 40.93 ± 0.06 | 2.93 ± 0.16 | 87.11 ± 1.46 | 72.88 ± 0.57 |
| Impregnation for 24 h | 39.17 ± 0.02 | 2.90 ± 0.05 | 84.43 ± 2.90 | 77.92 ± 4.30 |

Original corn stover represents the dry corn stover before impregnation with sulfuric acid solution. "Without impregnation, before pretreatment" represents corn stover impregnated by sulfuric acid solution. Each experiment was duplicated and indicated by standard deviation.

^a Determined after 72 h enzymatic hydrolysis.

^b Calculated as described by Zhang and Bao (2012).

Table 2

Composition of the soluble components of corn stover before and after pretreatment.

| | Glucose (mg/g DM) | Xylose (mg/g DM) | Acetic acid (mg/g DM) | Furfural (mg/g DM) | 5-HMF (mg/g DM) | O-Glu ^a (mg/g DM) | O-Xyl ^b (mg/g DM) |
|--|----------------------|---------------------|--------------------------|-----------------------|--------------------|---------------------------------|---------------------------------|
| <i>Corn stover before pretreatment</i> | | | | | | | |
| Without impregnation | 0.13 ± 0.13 | 0.11 ± 0.05 | 0.42 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.45 ± 0.09 | 1.01 ± 0.09 |
| Impregnation for 0.5 h | 0.14 ± 0.02 | 0.40 ± 0.23 | 0.57 ± 0.06 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.87 ± 0.03 | 1.09 ± 0.22 |
| Impregnation for 12 h | 0.82 ± 0.86 | 0.53 ± 0.43 | 0.56 ± 0.04 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.22 ± 0.30 | 1.42 ± 0.17 |
| Impregnation for 24 h | 0.37 ± 0.11 | 0.39 ± 0.12 | 0.59 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.57 ± 0.22 | 1.69 ± 0.29 |
| <i>Corn stover after pretreatment</i> | | | | | | | |
| Co-currently feeding mode | 13.51 ± 0.26 | 98.29 ± 0.58 | 10.43 ± 1.01 | 5.68 ± 0.37 | 2.25 ± 0.02 | 7.40 ± 1.34 | 24.21 ± 0.86 |
| Impregnation for 0.5 h | 14.22 ± 1.28 | 95.76 ± 1.51 | 9.82 ± 1.50 | 6.57 ± 0.34 | 2.26 ± 0.06 | 8.06 ± 5.10 | 18.73 ± 0.38 |
| Impregnation for 12 h | 10.10 ± 0.54 | 101.75 ± 0.15 | 9.10 ± 1.07 | 6.28 ± 0.14 | 1.75 ± 0.09 | 11.04 ± 4.06 | 28.65 ± 4.61 |
| Impregnation for 24 h | 10.71 ± 0.26 | 94.76 ± 1.25 | 9.22 ± 0.10 | 5.15 ± 0.02 | 1.83 ± 0.07 | 16.44 ± 0.55 | 27.22 ± 2.57 |

Corn stover was pretreated for 3 min at 185 °C using 2.5% sulfuric acid at 50 rpm agitation rate. Unit here was defined as micrograms components in the per gram dry corn stover.

^a O-Glu represents the oligomer of glucan.

^b O-Xyl represents the oligomer of xylan.

with increasing impregnation time (37.46% vs. 37.67%, 37.71%, and 38.38%), and hemicellulose (xylan) decreased slightly (20.92% vs. 20.14%, 20.88%, and 20.84%). Table 2 shows that the soluble components (glucose, xylose, acetic acid, and oligomers) also increased slightly, but the increases of glucose and xylose were faster than the other components. This result indicates that the prolonged impregnation did enhance the pre-hydrolysis of the virgin corn stover in a limited range. However, the differences in the composition of corn stover caused by impregnation almost disappeared after pretreatment: cellulose was 39.02% (without impregnation) vs. 40.17%, 40.93%, and 39.17% at different impregnation times (0.5, 12, and 24 h, respectively); xylan was 2.66% vs. 2.33%, 2.93%, and 2.90%. The soluble components also showed no differences with and without impregnation although all the insoluble increased significantly compared to that before pretreatment (Table 2). This result indicates that the co-currently feeding of corn stover and dilute acid solution with impregnation step was sufficient to ensure the high pretreatment efficiency under the helically agitated operation.

3.2. Impact of impregnation on the pretreatment in a non-agitated reactor

The pretreatment on the helically agitated reactor proved that impregnation had no obvious impact on the pretreatment efficiency. Then, the impact of impregnation on the pretreatment efficiency was examined when the helical agitation of the reactor was switched off. Fig. 1(b) shows the cellulose conversion ratio after enzymatic hydrolysis of the non-impregnated corn stover reached 71.56%, which was approximately 23% lower than that using corn stover after 12 h impregnation (87.74%). The ethanol yield after SSF using the pretreated corn stover materials with and without impregnation were 77.76% and 81.11%, respectively. A clear difference between the impregnation and non-impregnation appeared in the hydrolysis step when there was no agitation in the pretreatment reactor, indicating the mixing was important for co-currently feeding operation of the non-impregnated corn stover. The pretreatment efficiency of the non-impregnated corn stover was not sufficiently maintained under the statically pretreatment system.

3.3. SSF evaluation for pretreated corn stover with different impregnation mode

The SSF performance of the pretreated corn stover using the pretreated corn stover with and without impregnation was illustrated in Fig. 2. Fig. 2(a) shows that in the SSF of the corn stover pretreated without helical agitation, the final ethanol concentration reached

46.99 g/L and the yield reached 77.76% for non-impregnation, and the ethanol concentration were 46.50, 48.78, and 48.08 g/L and the yield were 75.32%, 81.11%, and 75.95% for impregnated for 0.5, 12, 24 h, respectively. The impregnation step seems to be less affected on ethanol fermentation comparing to the hydrolysis step, in which a significant difference caused by the impregnation step

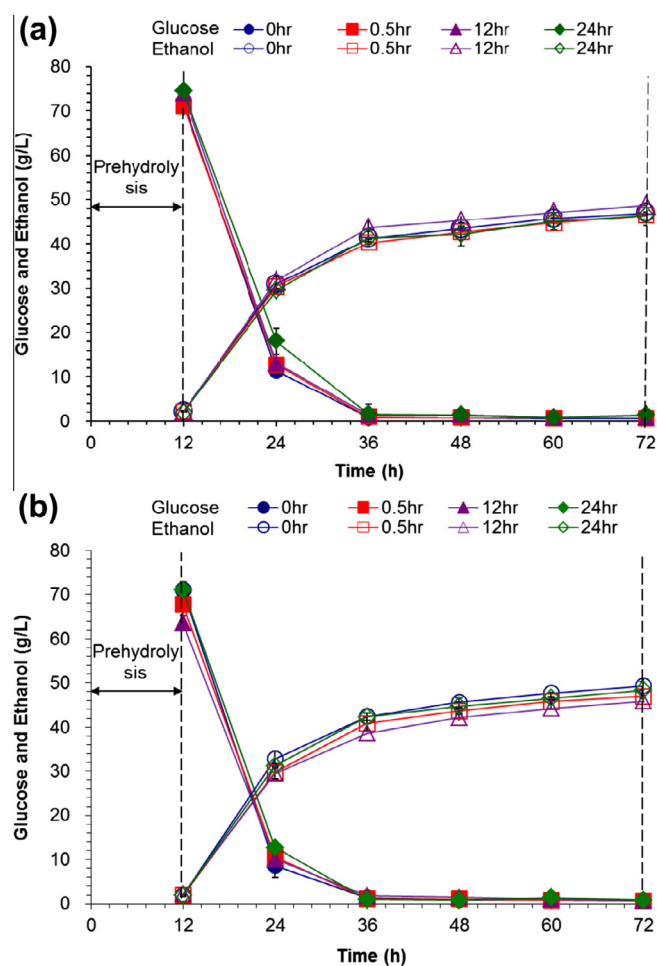


Fig. 2. SSF performance using the co-currently feeding CS and impregnated CS at different operation modes. (a) Pretreatment under no agitation; (b) pretreatment under helical agitation. SSF conditions: 25% solids loading, cellulase dosage of 15 FPU/g DM, 150 rpm, 10% (v/v) inoculum ratio; 50 °C, pH 4.8 at prehydrolysis step; 37 °C, pH 5.5 at SSF step.

was demonstrated. Similar trends were observed in the SSF using corn stover under the helical agitation as shown in Fig. 2(b). The final ethanol concentration reached 49.34, 46.91, 46.11, 48.36 g/L and the ethanol yield reached 79.80%, 73.87%, 72.88%, and 77.92% at non-impregnation, as well as impregnated for 0.5, 12, and 24 h, respectively.

4. Conclusion

Dry dilute acid pretreatment (DDAP) by co-currently feeding of corn stover and dilute sulfuric acid solution without impregnation was realized in the helically agitated reactor. The cellulose conversion and ethanol yield reached 84.77% and 79.80%, respectively, which were similar to those using well impregnated corn stover. When DDAP was carried out at non-agitated condition, the impregnation step led to the reduced conversion yield in the enzymatic hydrolysis step. This study provided a practical and useful way to reduce the cost and simplify the process of pretreatment.

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