COMBINED PRETREATMENT WITH WHITE-ROT FUNGUS AND ALKALI AT NEAR ROOM-TEMPERATURE FOR IMPROVING SACCHARIFICATION OF CORN STALKS

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Although biological pretreatment has the advantages of being environmentally friendly and having low-energy consumption, it usually requires a relatively long incubation time. In this study, a novel combined pretreatment with white-rot fungus and alkali at near room-temperature for saccharification of corn stalks was investigated to speed up the biological process. Biological pretreatment with *Irpex lacteus* or *Echinodontium taxodii* can improve enzymatic hydrolysis of corn stalk greatly, but the process requires a long time (60 days) to achieve a satisfactory sugar yield. The combination processes with the fungi were compared with the sole pretreatments. The results showed that the time of the biological process could be shortened to 15 days when the bio-treatment with *I. lacteus* was combined with alkali pretreatment. The efficiency of alkali pretreatment can be also enhanced significantly by biological treatment. 271.1mg/g of final glucose yield was obtained for the combination pretreatment, which was an improvement of 50.4% and 28.3% in comparison with the sole alkali pretreatment at the same and optimum reaction time, respectively. In conclusion, the combination of biological pretreatment with alkali processes not only speeded up the biological process, but also improved the sugar yield in comparison to the sole pretreatment and is favorable for the development of biological pretreatment.

Keywords: Combined pretreatment; Saccharification; White-rot fungi; Alkali; Room-temperature

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INTRODUCTION

Lignocellulosic biomass (LCB) offers a renewable, cost-effective, relatively greenhouse-gas-favorable, and broadly distributed material source of sugars that can be converted to biofuels and bioproducts by biorefinery processes (Kaparaju et al. 2009). Biorefinery of LCB usually involves the collection of biomass, deconstruction of cell wall polymers into component monomer sugars (pretreatment and saccharification), and conversion (fermentation) of sugars to products such as ethanol, methanol, and lactic acid (Sun and Cheng 2002). Pretreatment is needed before saccharification to overcome the LCB recalcitrance and to achieve high efficiency enzymatic hydrolysis (Himmel et al. 2007). Several thermochemical and biological pretreatment methods can be used for pretreatment of LCB (Chen et al. 2010; Yang and Wyman 2008). Thermochemical approaches, such as chemical and steam explosion pretreatment, offer high fermentable

sugar yield for biorefining, but these processes usually require a high temperature and operating pressure (Kumar et al. 2009; Lloyd and Wyman 2005; Stenberg et al. 2000). Currently, biological pretreatment has received considerable emphasis because of its advantages: being environmentally friendly and having a low energy consumption (Chen et al. 2010).

Biological pretreatment is a process of biological deconstruction of LCB using special microorganisms such as white-rot fungi that can degrade lignin efficiently (Hakala et al. 2004; Kirk and Farrell 1987). The enzymatic saccharification of LCB can be improved by fungal pretreatment due to the biodegradation of lignin and the increase of substrate porosity (Lee et al. 2007; Yu et al. 2009a; Zhang et al. 2007). Several white-rot fungi, such as *Pleurotus ostreatus*, *Irpex lacteus*, and *Echinodontium taxodii* have been used in biological pretreatments of LCB (Dias et al. 2010; Taniguchi et al. 2005; Yu et al 2009a). Despite the relatively high efficiency of delignification, most biological pretreatment processes have very low rates of use due to the restriction of the growth and reproduction rate of fungi. The biological pretreatment period of woods usually lasts 6 weeks or more to achieve satisfactory results (Hwang et al. 2008). Stalks are easier to deal with, but they still need 4 to 8 weeks of pretreatment (Taniguchi et al. 2005). Recently, some combined processes using thermochemical and biological pretreatment were applied for enzymatic hydrolysis of lignocelluloses in order to speed up the process. During the combined pretreatment process, the period of biological process can be shortened, and the severity of thermochemical reaction can become mild (Chen et al. 2010; Ma et al. 2010; Taniguchi et al. 2010). For example, Itoh et al. (2003) used white-rot fungi and ethanolysis to treat beech wood. They found that 1.6 times the ethanol was obtained after simultaneous saccharification and fermentation (SSF) by the combined pretreatment method when compared to sole ethanolysis. The fungal pretreatment can also save 15% of the electricity needed for the ethanolysis. Yu et al. (2009b) reported that the sugar yield could be elevated 30% after the combination pretreatment using hydrogen peroxide and *Pleurotus ostreatus*, and the biological pretreatment time could be reduced from 60 days to only 18 days.

Alkali pretreatment is a common thermochemical process in which LCB is treated with sodium hydroxide (NaOH), lime, or ammonia (Balat et al. 2008). This process causes less sugar degradation and is more effective in the conversion of crop residues such as corn stalk, wheat straw, and rice straw (Silverstein et al. 2007). Many of the caustic salts can be recovered or regenerated after alkali pretreatment. Silverstein et al. (2007) showed that NaOH pretreatment could significantly enhance the efficiency of cellulose saccharification in cotton stalks. In their studies they found that NaOH pretreatment achieved 60.97% hydrolysis, while the acid pretreatment only achieved 23.85%. NaOH pretreatment was found to cause a delignification reaction, a decrease in cellulose crystallinity, and an increase in surface area, resulting in the improvement of enzymatic hydrolysis (Wyman et al. 2005; Zhao et al. 2008). NaOH pretreatments are usually carried out at a high temperature (around 100 °C). Although alkaline delignification can occur at a low temperature, the rate of lignin degradation is very low. This makes it difficult to achieve satisfactory sugar yield by NaOH pretreatment at near room-temperature, which does not require special heating equipment and cuts the cost. Some studies showed that if alkali pretreatment is combined with microwave, hydrogen

peroxide, oxygen, or urea, the pretreatment efficiency could be improved (Bjerre et al. 1996; Rabelo et al. 2008). For example, with the assistance of microwave treatment, the delignification of wheat straw is highly improved during NaOH pretreatment, thus enhancing the efficiency of enzymatic hydrolysis and ethanol production (Zhu et al. 2006a,b).

In our previous research, it was found that the biological treatment with *Irpex lacteus* can enhance delignification during alkali pretreatment below 75 °C, which increases the enzymatic digestibility of glucan. However, the combination pretreatment process at room-temperature was not fully investigated (Yu et al. 2010). Thus, this study combines further the alkali pretreatment at near room-temperature (30 °C) with the biological treatment by *Irpex lacteus* and *Echinodontium taxodii*. This pretreatment was used for saccharification of corn stalks and to shorten the biological pretreatment time, also to fully evaluate the effects of biological treatment time and fungal species on the alkali pretreatment efficiency. The combination pretreatment, the sole biological pretreatment, and the sole alkali pretreatment were compared in order to identify the pretreatment that can provide the highest sugar yield on the basis of raw stalks.

EXPERIMENTAL

Microorganism and Inoculum

The white-rot fungi *Irpex lacteus* and *Echinodontium taxodii* were both isolated from the Shennong Nature Reserve (Hubei, China) (Xu et al. 2009; Yu et al. 2009a). The isolates were identified by rDNA internal transcribed spacer (ITS) sequence analysis, their GenBank accession numbers are FJ744594 (*I. lacteus*) and EF422215 (*E. taxodii*), respectively. The isolates were maintained on potato dextrose agar (PDA) slants at 4 °C.

Biological Pretreatment

Corn stalks are collected from Henan, air-dried, ground, and then passed through a 0.9 mm screen. White-rot fungal treatments were carried out in 250-mL Erlenmeyer flasks with 10 g cornstalks and 25 mL of distilled water. The flasks were sealed by plastic film and then sterilized at 121 °C for 30 min. The fungi were cultured in potato dextrose broth (PDB) medium at 28 °C for 7 days before the pretreatment. 5 mL of the fungal cultures were inoculated to the flasks. The incubated corn stalks were kept statically at 28 °C for 15, 30, 45, and 60 days, and then dried at 60 °C for 72 hours.

Alkali Pretreatment

The alkali pretreatments were carried out at near room-temperature (30 °C) because the experiments were carried out in the summer. The bio-treated corn stalks were subsequently treated with 0.25 M sodium hydroxide (NaOH) solution for 30 min after the 15-day or 30-day fungal treatment with *I. lacteus* or *E. taxodii*. The solid–liquid ratio was maintained at 1:15 (w:v). The raw corn stalks were used as controls. Corn stalks bio-treated by *I. lacteus* for 15 days were treated for 15-120 minutes respectively. All the pretreatments were carried out in triplicate. The residues were washed to neutral, dried at 60 °C for 72 hours, and weighed after the alkali pretreatment.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out with 2% substrate concentration in 50 mM sodium acetate buffer (pH 4.8) by cellulase (30 FPU/g substrate) at 50 °C. Cellulase was obtained from Sigma. After 1, 3, 6, 12, 24, and 48 hours the reaction mixture was interrupted by centrifugation at 10,000 rpm for 5 minutes, and the reducing sugar in the supernatant was determined according to the 3, 5-dinitrosalicyclic method (Behera et al. 1996). The hydrolysis yield of holocellulose was calculated as follows:

$$\text{Hydrolysis yield of holocellulose (\%)} = \frac{\text{Reducing sugar yield} \times 0.9 \times 100}{\text{Holocellulose content in pretreated corn stalk}}$$

The glucose in the hydrolyzate was also determined by a high performance liquid chromatography (HPLC) system (Agilent 1200, USA) with sugar-pak-1 column (Waters, China) and the refractive index (RI) detector (Agilent G1362A, USA). The mobile phase was deionized water. The column was used at 75 °C with a flow rate of 0.6 mL/min.

Chemical Analysis of Corn Stalks

Lignin, cellulose, and hemicellulose contents in all samples were determined according to procedures of ‘‘determination of structural polysaccharides and lignin in biomass” (Version 2006) from the National Renewable Energy Laboratory (NREL). Lignin content was calculated as the sum of acid soluble lignin (ASL) and acid insoluble lignin (AIL) content. Cellulose content was defined as glucan content and hemicellulose was defined as the sum of xylan and arabinocan content in samples. Holocellulose content was defined as the sum of cellulose and hemicellulose contents. Xylan, arabinocan, and glucan content were calculated by the yield of arabinose, xylose and glucose, multiplied by the coefficients 0.88, 0.88, and 0.9, respectively.

RESULTS AND DISCUSSION

Biological Pretreatment of Corn Stalks

Figures 1a and 1b show the reducing sugar yield of corn stalks treated with white-rot fungi, *Irpex lacteus*, and *Echinodontium taxodii* after enzymatic hydrolysis. As expected, the reducing sugar yield increased greatly as the pretreatment time increased. The yield reached 366.3 mg/g raw corn stalks for *I. lacteus* and 300.5 mg/g for *E. taxodii* after 60-day pretreatment, which were 2.9 and 2.2 times the reducing sugar yield of non-treated corn stalks, respectively. The increase of reducing sugar yield was attributed to the decrease in the natural recalcitrance of corn stalks to enzymatic hydrolysis. As shown in Figs. 1c and 1d, the maximum hydrolysis yield of corn stalk holocellulose can reach 71.0% of theoretical yield for *I. lacteus* and 48.6% of theoretical yield for *E. taxodii* after 60-day pretreatment, which were 5.2 and 3.2 times that of the raw material, respectively.

I. lacteus reduced the recalcitrance of corn stalk more effectively than *E. taxodii*. With the prolongation of the biological pretreatment time, the recalcitrance of corn stalks can be weakened, but the polysaccharide loss would be increased during the pretreatment. Only 64.3% of cellulose was recovered after 60-day pretreatment with *I. lacteus*.

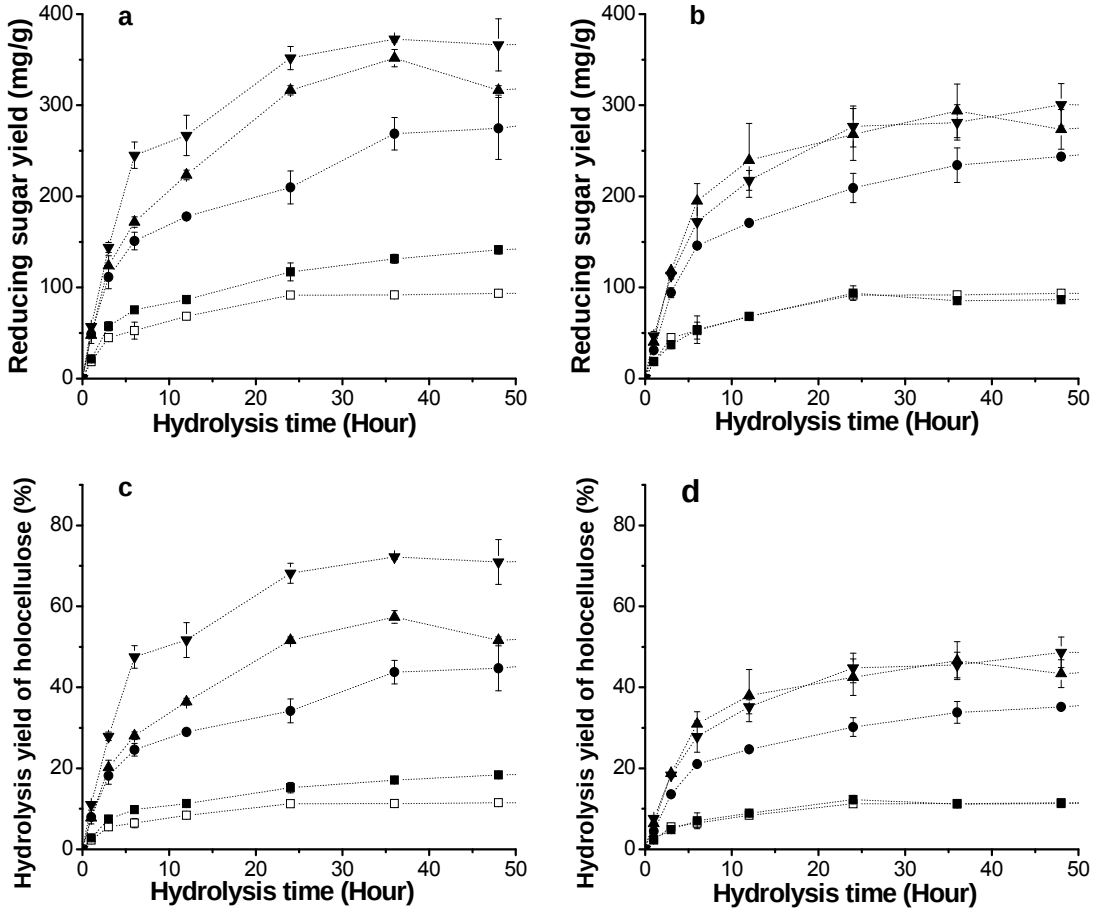

Fig. 1. Reducing sugar yield and hydrolysis yield of holocellulose after enzymatic hydrolysis of corn stalks pretreated by *Irpex lacteus* (a, c) and *Echinodontium taxodii* (b, d) for 0 d (■), 15 d (□), 30 d (●), 45 d (▲)，60d (▼)

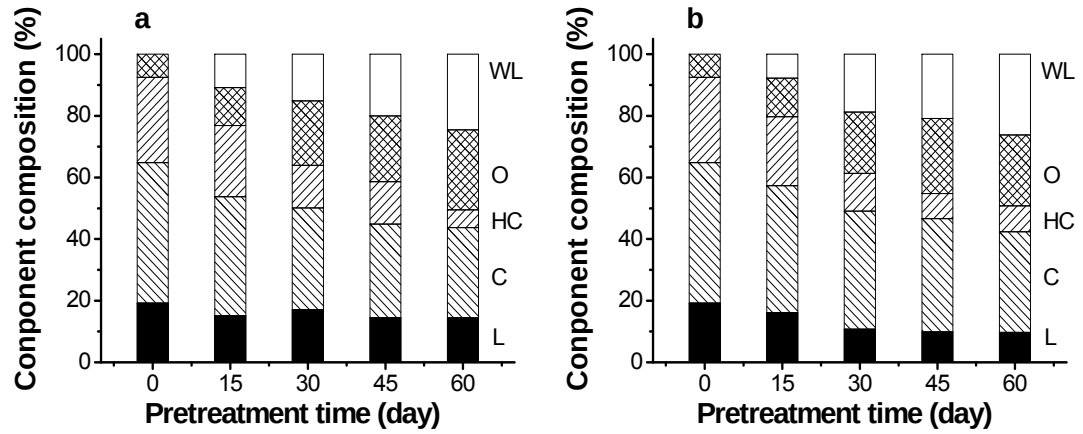

Fig. 2. The changes of corn stalk components during bio-pretreatment with *Irpex lacteus* (a) and *Echinodontium taxodii* (b). L: weight loss, O: other, HC: hemicellulose, C:cellulose, L: lignin

Although 60-day pretreatment with *I. lacteus* can improve the hydrolysis efficiency greatly, the loss of polysaccharide would lower the final reducing sugar yield. *E. taxodii* showed higher selective lignin-degrading ability than *I. lacteus* (Fig. 2b). As a consequence, even though *E. taxodii* is less efficient in weakening the lignocellulose recalcitrance when compared to *I. lacteus*, the polysaccharide recovered from the pretreatment with *E. taxodii* is more than that from the pretreatment with *I. lacteus*. This trade-off contributes to narrowing the gap in final reducing sugar yield between the two fungal pretreatments. Thus, a long-time bio-treatment should lead to a low efficiency of the pretreatment process due to high sugar loss.

Evaluation of Bio-pretreatment Combined with Alkali Pretreatment

In order to enhance the efficiency of biological pretreatment, the combined pretreatment was evaluated in the study. In the combined process, corn stalks were first pretreated by white-rot fungus, *I. lacteus* or *E. taxodii*, and then treated with NaOH solution at near room-temperature (30 °C). Figure 3 shows that the reducing sugar yield of corn stalks was only 303.9 mg/g after the sole alkali pretreatment for 30 min. The efficiency of NaOH pretreatment is usually decreased when the reaction temperature decreases, which might be caused by incomplete delignification (Silverstein et al. 2007). Thus, few studies have used NaOH pretreatment at room-temperature to improve the saccharification of lignocellulose, even though the pretreatment at room-temperature does not require special heating equipment and is easy to carry out. However, the reducing sugar yield was significantly improved when the biological pretreatment process with *I. lacteus* was combined with the alkali process in this study. This finding indicated that the bio-treated substrate reacted with NaOH more easily under mild conditions than the raw substrate. Some studies showed that using fungal treatment before the thermochemical pretreatment can lower process severity by improving solvent accessibility or reaction rate, but not all fungi contribute to the sequent thermochemical reaction (Monrroy et al. 2010; Itho et al. 2003; Yu et al. 2009a). As shown in Fig. 3, the pretreatment with *E. taxodii* did not have much effect on the NaOH pretreatment at near room-temperature. Therefore, *I. lacteus* was the more promising fungus for combined pretreatment rather than *E. taxodii*.

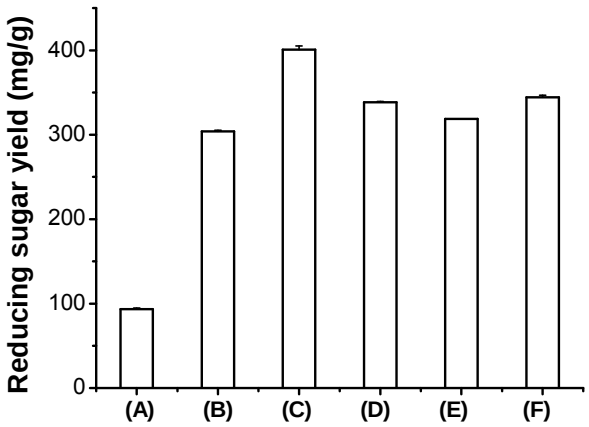

Fig. 3. Comparison of reducing sugar yield of corn stalks after the sole alkali pretreatment (B) and after combined pretreatment with *I. lacteus* for 15 d (C) and 30 d (D), also with *E. taxodii* for 15 d (E) and 30 d (F). Condition (A) denotes raw corn stalk.

The efficiency of alkali pretreatment could be improved considerably by biological pretreatment for only 15 days. The final reducing sugar yield could reach 400.1 mg/g after combined pretreatment (Fig. 3), which was an increase of 31.7% when compared to the sole alkali pretreatment. Meanwhile, the reduction of biological pretreatment time may be responsible for the increase in recovery of polysaccharide after the pretreatment. With respect to the sole bio-pretreatment with *I. lacteus*, prolonging the bio-pretreatment time to 30 days resulted in a decrease in the reducing sugar yield to only 338.5 mg/g (Fig. 3), which might have been a result of the increase in sugar loss during bio-pretreatment. Thus, the 15-day pretreatment with *I. lacteus* was chosen to be combined with alkali pretreatment.

Combined Pretreatment with *I. lacteus* and NaOH

To investigate the effect of combined pretreatment on sugar yield further, corn stalks were treated by *I. lacteus* for 15 days and then treated by NaOH at near room-temperature for various time periods. The reducing sugar yields of all samples after the sole alkali pretreatment increased progressively with the prolongation of the pretreatment time and became almost constant at 90 minutes of enzymatic hydrolysis. However, in the case of the combined pretreatment, the time of constant yield was as early as 45 minutes, which means that the rate of alkali pretreatment could be improved by the biological pretreatment (Fig. 4a, b). The final reducing sugar yield of corn stalks after the combined pretreatment and alkali pretreatment, respectively, was 458.7 mg/g and 425.7 mg/g at an alkali pretreatment time of 120 minutes. Reducing sugar yield improved 7.8% by using combined pretreatment as compared to using sole alkali pretreatment.

As expected, the combination pretreatment can also lower the recalcitrance of corn stalks to hydrolysis more significantly than the sole alkali pretreatment. After enzymatic hydrolysis, 82% of the holocellulose from corn stalk pretreated with the combined method was hydrolyzed at an alkali process time of 120 minutes, but only 61% was hydrolyzed for the sole alkali pretreatment (Fig. 4c, d). When the alkali process of combined pretreatment was only 15 minutes long, the hydrolysis yield of holocellulose was 67.3%, which was still higher than the maximum hydrolysis yield of holocellulose after the sole alkali pretreatment. To summarize, the biological pretreatment with *I. lacteus* can improve the efficiency of NaOH pretreatment significantly at near room-temperature.

Comparison of Combined Pretreatment and Sole Pretreatment

The final reducing sugar yield was affected by both the efficiency of enzymatic hydrolysis and the total sugar loss during the pretreatment (Chen et al. 2010). As shown in Fig. 3, the improvement in final reducing sugar yield was far less significant than that in hydrolysis yield of holocellulose, as the additional biological pretreatment process could cause extra sugar loss. Thus, it is necessary to evaluate sugar transfer during the whole pretreatment process in order to identify the best pretreatment process.

The sugar transfer of following processes are compared in Table 1: (1) the combination pretreatment of 15-day biological treatment with *I. lacteus* and 45-min alkali pretreatment, (2) the sole biological pretreatment for 60 days, and (3) the sole alkali pretreatment for 45 minutes and (4) for 120 minutes.

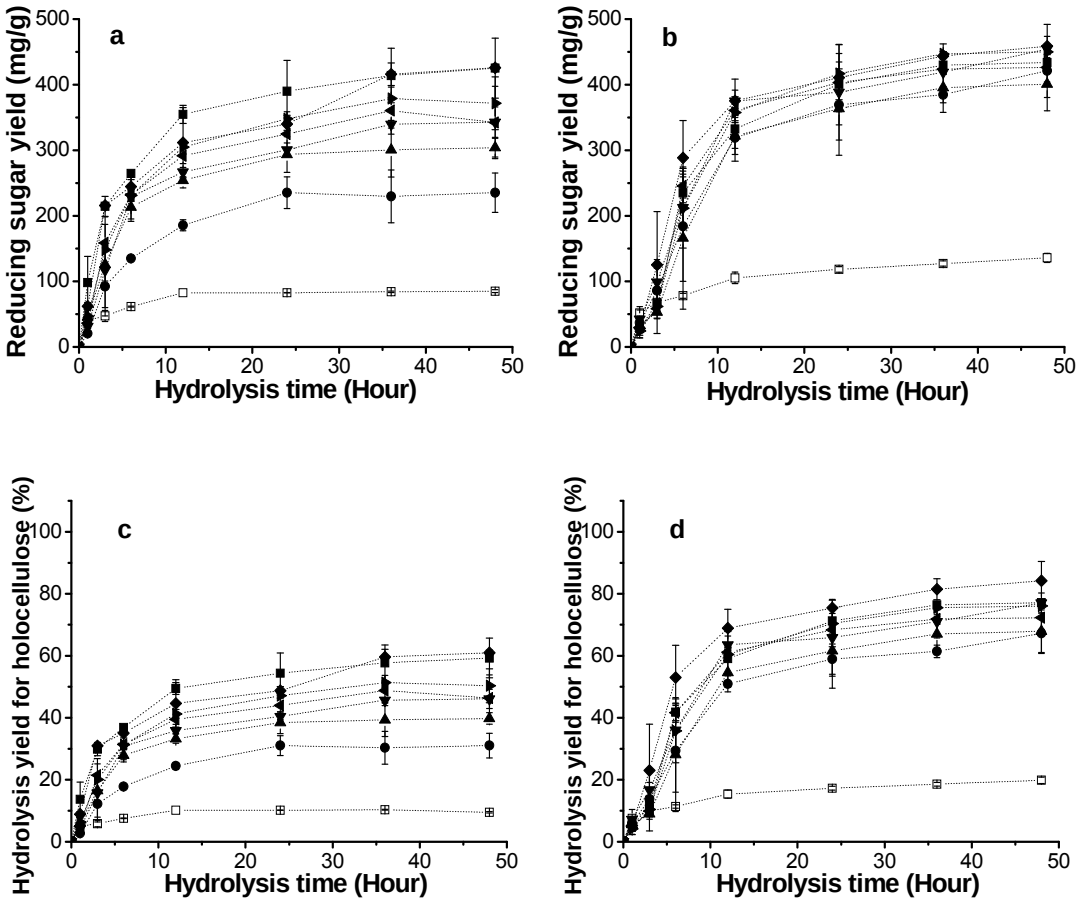

Fig. 4. Reducing sugar yield of corn stalks pretreated by the alkaline pretreatment (a, c) and combined pretreatment (b, d) with *Irpex lacteus* and alkali. The alkaline pretreatment was carried out at 30 °C for 0 min (□), 15 min (●), 30 min (▲), 45 min (▼), 60 min (◄), 75 min (►), 90 min (■), and 120 min (◆).

It was obvious that the maximum glucan content in the enzymatic hydrolyzate was obtained through combined pretreatment. Compared to the sole alkali pretreatment, the combined pretreatment caused higher xylan loss. One part of the loss was attributed to the biological processes, while another part resulted from the enhanced alkali degradation of xylan by biological process, which also contributes to improving the hydrolysis of glucan (Sun and Cheng 2002). The hydrolysis yield of glucan in the pretreated substrate for the combined pretreatment was higher (72%) than that for the sole alkali pretreatment (41% and 52%), but was lower than that for the sole biological pretreatment (81%). This indicated that the biological process was more effective in lowering the lignocellulose recalcitrance than the combined process, but a long-time treatment process caused great sugar loss, which was responsible of the decrease of the final sugar yield.

Although the 15-day biological treatment in combination pretreatment caused extra sugar loss (69.5 and 43.7 mg/g for glucan and xylan, respectively) , the amount of sugar loss was much smaller than that caused by the 60-day biological treatment (162.2 and 187.5 mg/g for glucan and xylan, respectively). The negative effect could be made up

by the significant enhancement of the enzymatic hydrolysis yield after the combined pretreatment. As a result, glucose yield of corn stalks via combined pretreatment was improved by 50.4% when compared to the sole alkali pretreatment under the same condition (45 minutes). Even when the time of sole alkali pretreatment was 120 minutes, there was a 28.3% increase in glucose yield via combined pretreatment.

Table 1. Sugar Transfer during Sole Pretreatment and Combination Pretreatment *

	Pretreatment method	Amount of sugar In 1 g of corn stalks (mg)	Amount of sugar in the solid residue after pretreatment (mg)		Amount of sugar in the Enzymatic hydrolyzate (mg)
			Biological pretreatment	Alkali pretreatment	
Glucan	1	455.2(11.2)	385.7(3.6)	374.1(7.5)	271.1(5.1)
	2		292.7(4.6)		237.3(2.4)
	3			436.8(1.3)	180.3(1.7)
	4			404.5(6.9)	211.3(10.8)
Xylan	1	245.0(1.7)	201.3(2.1)	136.0(2.7)	95.0(3.1)
	2		57.5(3.3)		27.0(1.9)
	3			199.2(0.3)	88.3(2.6)
	4			191.9(4.2)	116.0(1.8)
* Numbers in parentheses represent standard deviations.					

In recent years, some techniques of combination pretreatment have been applied to speed up the biological treatment process. Muñoz et al. (2007) combined the biological pretreatments using *Ceriporiopsis subvermispora* and *Ganoderma australe* with an organosolv process for enzymatic hydrolysis of *Pinus radiata* wood. The biological pretreatment requires only 30 days, and glucan conversion of the pretreated wood can reach about 100%, but the process requires a reaction temperature of 200 °C. Yu et al. (2009b) reduced the time of biological pretreatment with *Pleurotus ostreatus* from 60 days to 18 days for enzymatic hydrolysis of rice hull by combining hydrogen peroxide treatment and biological treatment at 25 °C. The combination pretreatment leads to 49.6% of net glucose yield (220.4 mg of glucose produced in enzymatic hydrolyzate for 1 g of raw substrate). Baba et al. (2011) applied the combined process by solvolysis at 200 °C and cultivation with a new fungal isolate (*Phellinus sp.*) to develop a pretreatment process of recalcitrant softwood (Japanese cedar). The biological pretreatment requires 56 days and total reducing sugars yield reaches 422 mg for 1 g of the bio-pretreated softwood. Compared with these combination processes, combined pretreatment with white-rot fungus and alkali at near room-temperature cut down the time needed by biological pretreatment more significantly, led to higher efficiency of saccharification, or had milder pretreatment conditions. According to the comprehensive comparison, the combination of biological pretreatment and NaOH pretreatment at near room-temperature is an attractive process for the pretreatment of corn stalks.

CONCLUSIONS

1. A long-time (60 days) biological pretreatment with *Irpex lacteus* and *Echinodontium taxodii* can improve the reducing sugar yield greatly for the enzymatic hydrolysis of corn stalk, but leads to high sugar loss during the pretreatment.
2. When the bio-treatment with *I. lacteus* is combined with NaOH pretreatment at near room-temperature, the time of biological process was shortened to 15 days and the efficiency of NaOH pretreatment was enhanced.
3. The combined pretreatment process is favorable for further development and application of biological pretreatment.

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REFERENCES CITED

Balat, M., Balat, H., and Öz, C. (2008). "Progress in bioethanol processing," *Prog. Energ. Combust. Sci.* 34(5), 551-573.

Baba, Y., Tanabe, T., Shirai, N., Watanabe, T., Honda, Y., and Watanabe, T. (2011). "Pretreatment of Japanese cedar wood by white rot fungi and ethanolysis for bioethanol production," *Biomass Bioenerg.* 35(1), 320-324.

Behera, B. K., Arora, M., and Sharma, D. K. (1996). "Scanning electron microscopic (SEM) studies on structural architecture of lignocellulosic materials of *Calotropis procera* during its processing for saccharification," *Bioresource Technol.* 58(3), 241-245.

Bjerre, A. B., Olesen, A. B., Fernqvist, T., Ploger, A., and Schmidt, A. S. (1996). "Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose," *Biotechnol. Bioeng.* 49(5), 568-577.

Chen, S., Zhang, X., Singh, D., Yu, H., and Yang, X. (2010). "Biological pretreatment of lignocellulosics: Potential, progress and challenges," *Biofuels* 1(1), 177-199.

Dias, A. A., Freitas, G. S., Marques, G. S. M., Sampaio, A., Fraga, I. S., Rodrigues, M. A. M., Evtuguin, D. V., and Bezerra, R. M. F. (2010). "Enzymatic saccharification of biologically pre-treated wheat straw with white-rot fungi," *Bioresource Technol.* 101(15), 6045-6050.

Hakala, T. K., Maijal, P., Konn, J., and Hatakka, A. (2004). "Evaluation of novel wood-rotting polypores and corticioid fungi for the decay and biopulping of Norway spruce (*Picea abies*) wood," *Enzyme Microb. Tech.* 34(3-4), 255-263.

Himmel, M. E., Ding, S., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 315(5813), 804-807.

Hwang, S., Lee, S., Kim, H., Ka, J., Kim, K., and Song, H. (2008). "Biodegradation and saccharification of wood chips of *Pinus strobus* and *Liriodendron tulipifera* by white rot fungi," *J. Microbiol. Biotechnol.* 18(11), 1819-1826.

Itoh, H., Wada, M., Honda, Y., Kuwahara, M., and Watanabe, T. (2003). "Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi," *J. Biotechnol.* 103(3), 273-280.

Kaparaju, P., Serrano, M., Thomsen, A. B., Kongjan, P., and Angelidaki, I. (2009). "Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept," *Bioresource Technol.* 100, 2562-2568.

Kirk, T. K., and Farrell, R. L. (1987). "Enzymatic "combustion": The microbial degradation of lignin," *Annu. Rev. Microbiol.* 41, 465-505.

Kumar, R., Mago, G., Balan, V., and Wyman, C. E. (2009). "Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies," *Bioresource Technol.* 100(17), 3948-3962.

Lee, J. (1997). "Biological conversion of lignocellulosic biomass to ethanol," *J. Biotechnol.* 56(1), 1-24.

Lee, J., Gwak, K., Park, J., Park, M., Choi, D., Kwon, M., and Choi, I. (2007). "Biological pretreatment of softwood *Pinus densiflora* by three white rot fungi," *J. Microbiol.* 45, 485-491.

Lloyd, T. A., and Wyman, C. E. (2005). "Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids," *Bioresource Technol.* 96(18), 1967-1977.

Ma, F., Yang, N., Xu, C., Yu, H., Wu, J., and Zhang, X. (2010). "Combination of biological pretreatment with mild acid pretreatment for enzymatic hydrolysis and ethanol production from water hyacinth," *Bioresource Technol.* 101(24), 9600-9604.

Monrroy, M., Ibañez, J., Melin, V., Baeza, J., Mendonça, R. T., Contreras, D., and Freer, J. (2010). "Bioorganosolv pretreatments of *P. radiata* by a brown rot fungus (*Gloephyllum trabeum*) and ethanolysis," *Enzyme Microb. Tech.* 47(1-2), 11-16.

Muñoz, C., Mendonca, R., Baeza, J., Berlin, A., Saddler, J., and Freer, J. (2007). "Bioethanol production from bio-organosolv pulps of *Pinus radiata* and *Acacia dealbata*," *J. Chem. Technol. Biot.* 82(8), 767-774.

Rabelo, S., Filho, R., and Costa, A. (2008). "A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production," *Appl. Biochem. Biotech.* 144(1), 87-100.

Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., Boyette, M. D., and Osborne, J. (2007). "A comparison of chemical pretreatment methods for improving saccharification of cotton stalks," *Bioresource Technol.* 98, 3000-3011.

Stenberg, K., Bollok, M., Reczey, K., Galbe, M., and Zacchi, G. (2000). "Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production," *Biotechnol. Bioeng.* 68(2), 204-10.

Sun, Y., and Cheng, J. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresource Technol.* 83(1), 1-11.

Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K., and Tanaka, T. (2005). "Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw," *J. Biosci. Bioeng.* 100(6), 637-643.

Taniguchi, M., Takahashi, D., Watanabe, D., Sakai, K., Hoshino, K., Kouya, T., and Tanaka, T. (2010). "Effect of steam explosion pretreatment on treatment with *Pleurotus ostreatus* for the enzymatic hydrolysis of rice straw," *J. Biosci. Bioeng.* 110(4), 449-452.

Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresource Technol*. 96(6), 673-686.

Xu, C., Ma, F., and Zhang, X. (2009). "Lignocellulose degradation and enzyme production by *Irpex lacteus* CD2 during solid-state fermentation of corn stover," *J. Biosci. Bioeng*. 108(5), 372-375.

Yang, B., and Wyman, C. E. (2008). "Pretreatment: The key to unlocking low-cost cellulosic ethanol," *Biofuels Bioprod. Bioref*. 2(1), 26-40.

Yu, H., Du, W., Zhang, J., Ma, F., Zhang, X., and Zhong, W. (2010). "Fungal treatment of cornstalks enhances the delignification and xylan loss during mild alkaline pretreatment and enzymatic digestibility of glucan," *Bioresource Technol*. 101(17), 6728-6734.

Yu, H., Guo, G., Zhang, X., Yan, K., and Xu, C. (2009a). "The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods," *Bioresource Technol.* 100(21), 5170-5175.

Yu, J., Zhang, J., He, J., Liu, Z., and Yu, Z. (2009b). "Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull," *Bioresource Technol.* 100(2), 903-908.

Zhang, X., Yu, H., Huang, H., and Liu, Y. (2007). "Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms," *Int. Biodeter. Biodegr.* 60(3), 159-164.

Zhao, X., Zhang, L., and Liu, D. (2008). "Comparative study on chemical pretreatment methods for improving enzymatic digestibility of crofton weed stem," *Bioresource Technol.* 99(9), 3729-3736.

Zhu, S., Wu, Y., Yu, Z., Chen, Q., Wu, G., Yu, F., Wang, C., and Jin, S. (2006a). "Microwave-assisted alkali pre-treatment of wheat straw and its enzymatic hydrolysis," *Biosystems Eng*. 94(3), 437-442.

Zhu, S., Wu, Y., Yu, Z., Zhang, X., Wang, C., Yu, F., and Jin, S. (2006b). "Production of ethanol from microwave-assisted alkali pretreated wheat straw," *Process Biochem.* 41(4), 869-873.

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