

# Enzymatic Digestibility of Corn Stover Fractions in Response to Fungal Pretreatment

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**ABSTRACT:** Corn stover fractions (leaves, cobs, and stalks) were studied for enzymatic digestibility after pretreatment with a white rot fungus, *Ceriporiopsis subvermispota*. Among the three fractions, leaves had the least recalcitrance to fungal pretreatment and the lignin degradation reached 45% after 30 days of pretreatment. The lignin degradation of stalks and cobs was similar but was significantly lower than that of leaves ( $p < 0.05$ ). For all fractions, xylan and glucan degradation followed a pattern similar to lignin degradation, with leaves having a significantly higher percentage of degradation ( $p < 0.05$ ). Hydrolytic enzyme activity also revealed that the fungus was more active in the degradation of carbohydrates in leaves. As a result of fungal pretreatment, the highest sugar yield, however, was obtained with corn cobs.

## 1. INTRODUCTION

Currently, the majority of ethanol production in the U.S. is based on corn grain, which supplied about 10 billion gallons in 2009.<sup>1</sup> The supply of corn ethanol is expected to be limited in the near future because of competition with feed and food production.<sup>1</sup> Ethanol production from lignocellulosic biomass, which is rich in carbohydrates and is widely available, is becoming technically and economically feasible due to increased investment in research and development. According to the “Billion-Ton Annual Supply” report, more than 1.3 billion tons of biomass could be available annually from agricultural and forestry sectors in the U.S., which is enough to produce fuel ethanol to meet 30% of current gasoline consumption.<sup>2</sup>

Corn stover, typically composed of 35–40% cellulose, 20–25% hemicellulose, and 15–20% lignin,<sup>3</sup> is the most abundant agricultural residue in the U.S., with availability estimated at 170–256 million tons annually.<sup>2</sup> The domestic availability and high carbohydrate content of corn stover have made it a prospective substrate for cellulosic ethanol production. However, native corn stover is recalcitrant to hydrolytic enzyme attack due to cellulose crystallinity, limited accessible surface area, and covalent bonds between lignin and hemicellulose.<sup>4</sup> Therefore, various pretreatment methods, such as acid hydrolysis and steam explosion, have been used for reducing biomass recalcitrance in order to improve enzymatic hydrolysis. Compared to thermo-chemical pretreatments, microbial pretreatment via solid-state fermentation has advantages including, but not limited to, simple techniques, low energy requirements, no or reduced output of waste stream, reduced downstream processing costs, and no or less inhibitors to ethanol fermentation.<sup>5</sup> Some previous studies have demonstrated that pretreatment of corn stover by white rot fungi significantly improved enzymatic hydrolysis.<sup>5–7</sup>

The structure and composition of corn stover varies considerably throughout the growing season and between various fractions of the plant.<sup>8</sup> These differences determine optimal pretreatment conditions, enzymatic digestibility, and

fermentable sugar yield potential.<sup>8</sup> In general, less recalcitrant plant fractions require less severe pretreatment and give higher sugar release. Therefore, separate pretreatment of the different fractions of corn stover, such as leaves, cobs, and stalks, could be an alternative for reducing pretreatment and hydrolysis costs.<sup>9</sup>

From the view of sustainable harvesting, corn stover collection for maximizing ethanol production must be balanced with soil erosion control. Considering this need for balance, several studies have focused on the optimization of harvest scenarios based on combined pretreatment and enzymatic hydrolysis.<sup>8,10–12</sup> For example, Garlock et al.<sup>8</sup> proposed that the optimal harvest order for the selective harvest of corn stover would be husks followed by leaves, then stalks, and, at last, cobs. While thermal/chemical pretreatment (e.g., AFEX and NaOH soaking) of separate corn fractions has been evaluated,<sup>8,10</sup> the variance in sugar yield for each fraction via fungal pretreatment has not been evaluated.

In this study, pretreatment with *Ceriporiopsis subvermispota*, a white rot fungus, followed by enzymatic hydrolysis was performed on three corn stover fractions (leaves, stalks, and cobs). The objectives were to (1) determine fungal degradation of the individual corn stover fraction; (2) investigate the cell wall degrading enzymes involved in fungal pretreatment; and (3) evaluate enzymatic hydrolysis sugar yields of fungal pretreated corn stover fractions.

## 2. MATERIALS AND METHODS

**2.1. Corn Stover.** Corn stover (baled) was collected during the harvesting season from the farm of the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH, USA. After oven-drying at 40 °C for 72 h, the corn stover

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was manually separated into three fractions (leaves, cobs, and stalks). Each fraction was ground separately through a 5 mm screen and then stored in airtight containers at room temperature before use.

**2.2. Fungus and Inoculation.** *C. subvermispora* (ATCC 96608) was purchased from American Type Culture Collection (Manassas, VA, USA) and then maintained on malt extract media plus glycerin at  $-20\text{ }^{\circ}\text{C}$ . The fungus was revived in malt extract agar (2% MEA, w/v) and, subsequently, grown in 50 mL of liquid media (2% malt extract, w/v) in a 500 mL cotton-plugged Erlenmeyer flask. After stationary incubation at  $28\text{ }^{\circ}\text{C}$  for 7 d, the liquid culture was collected, aseptically homogenized in a blender for three 15 s cycles, and then used as inoculation for fungal pretreatment.

**2.3. Fungal Pretreatment.** A 10 g portion of each corn stover fraction was mixed with deionized water to obtain an optimal moisture content of 75%, and this mixture was autoclaved in 250 mL Erlenmeyer flasks at  $121\text{ }^{\circ}\text{C}$  for 15 min. After being allowed to cool to room temperature, the flasks were inoculated with 2 mL of homogenized liquid culture and sealed with a cotton plug. Fungal treatment was carried out in an incubator at  $28\text{ }^{\circ}\text{C}$  under static conditions for 35 d. At predetermined time periods (day 2, 4, 7, 10, 13, 17, 21, 25, 30, and 35), all contents in the flask were removed and then mixed thoroughly to ensure homogeneity for compositional analysis and enzyme activity assay. Samples of the corn stover fractions without fungal inoculation were used as controls. All tests were performed in duplicate.

**2.4. Enzymatic Hydrolysis.** Enzymatic hydrolysis experiments were conducted following NREL Laboratory Analytical Procedures (LAP).<sup>13</sup> Cellulase (Spezyme CP) was obtained from Genencor (Palo Alto, CA, USA) and had a cellulase activity of 50 FPU/mL. Samples of corn stover fractions were loaded at 2.5% (w/w) into a citrate buffer (0.05 M, pH 4.8) and cellulase was dosed at 10 FPU/g solid on a dry basis. After hydrolyzing at  $50\text{ }^{\circ}\text{C}$  for 72 h in an incubator shaker, hydrolysis was terminated by boiling the hydrolyzed slurry for 5 min in order to inactivate the cellulase. After cooling, the supernatant was collected and filtered for high performance liquid chromatograph (HPLC) analysis. Glucose and xylose yields were defined as the percentage of the corresponding theoretical sugar yield of each untreated corn stover fraction.

**2.5. Enzyme Assay.** Crude enzymes were obtained by extracting the fungal treated corn stover fractions with sodium acetate buffer (50 mM, pH 4.5) in an incubator shaker (150 rpm and  $28\text{ }^{\circ}\text{C}$ ). After 4 h, the liquid fraction was filtered and collected for enzyme assay. Manganese peroxidase (MnP) activity was assayed by phenol red oxidation<sup>14</sup> and laccase activity by oxidation of 2,2-azino-bis-(3-ethyl benzthiazoline-6-sulfonate).<sup>15</sup> The CMC activity of cellulase (endo- $\beta$ -1,4-glucanase) was measured with carboxymethyl cellulose (CMC) as the substrate.<sup>16</sup> Birchwood xylan (Sigma-Aldrich) was used as the substrate for the measurement of xylanase.<sup>17</sup> The detailed assay procedure was described in our previous study.<sup>6</sup>

**2.6. Analytical Methods.** The feedstock composition was analyzed following NREL Laboratory Analytical Procedure (LAP).<sup>18,19</sup> HPLC (Shimadzu LC-20 AB, Columbia, MD, USA) equipped with a Bio-Rad Aminex HPX-87P column and a refractive index detector (RID) was used to analyze monomeric sugars. Temperatures of the column and the RID were maintained at  $80$  and  $55\text{ }^{\circ}\text{C}$ , respectively. HPLC-grade water was used as the mobile phase, eluting at a flow rate of 0.6

mL/min. Lignin S/G ratios were determined by pyrolysis molecular beam mass spectroscopy (pyMBMS) according to the method described by Sykes et al.<sup>20</sup> Cellulose and hemicellulose-derived sugar contents were calculated from their corresponding monomers.<sup>18</sup> Degradation of lignin, glucan, and xylan was defined as the loss of the corresponding component during fungal pretreatment on the basis of the starting materials.

**2.7. Statistical Analysis.** SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) was used for analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were run on all experimental results (e.g., biomass composition, degradation, sugar yield) with a threshold  $p$ -value of 0.05.

### 3. RESULTS AND DISCUSSION

**3.1. Composition of Corn Stover Fractions.** Compositions of three major fractions of corn stover are presented in Table 1. The cellulose contents were similar in leaves and cobs,

**Table 1. Compositions (%) of Fractions of Corn Stover<sup>a</sup>**

composition	leaves	stalks	cobs
holocellulose	53.0 $\pm$ 1.3	59.3 $\pm$ 1.0	60.3 $\pm$ 0.7
–cellulose	33.8 $\pm$ 0.7	40.7 $\pm$ 0.5	34.1 $\pm$ 0.1
–hemicellulose	19.1 $\pm$ 0.6	18.5 $\pm$ 0.5	26.2 $\pm$ 0.5
–xylan	17.1 $\pm$ 0.6	16.4 $\pm$ 0.5	24.0 $\pm$ 0.5
–galactan	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
–arabinoxylan	2.0 $\pm$ 0.0	2.1 $\pm$ 0.1	2.3 $\pm$ 0.0
lignin	18.4 $\pm$ 0.5	23.0 $\pm$ 0.2	18.5 $\pm$ 0.5
–acid-insoluble lignin	16.5 $\pm$ 0.5	21.4 $\pm$ 0.2	16.0 $\pm$ 0.3
–acid-soluble lignin	1.9 $\pm$ 0.0	1.6 $\pm$ 0.1	2.5 $\pm$ 0.1
extractives	11.5 $\pm$ 0.8	9.9 $\pm$ 0.7	5.1 $\pm$ 0.4
–water extractives	9.5 $\pm$ 0.3	6.2 $\pm$ 0.4	3.4 $\pm$ 0.4
–ethanol extractives	2.1 $\pm$ 0.5	3.7 $\pm$ 0.3	1.6 $\pm$ 0.0
ash	8.3 $\pm$ 0.6	2.8 $\pm$ 0.1	2.1 $\pm$ 0.2
others	8.9 $\pm$ 3.2	5.1 $\pm$ 2.0	14.0 $\pm$ 3.4

<sup>a</sup>The numbers after “ $\pm$ ” are standard errors for each mean.

but stalks had the highest cellulose content. Cobs are generally rich in xylan and, thus, possessed significantly higher xylan content than leaves and stalks ( $p < 0.05$ ). Acid soluble lignin content in cobs was also higher than the other two fractions ( $p < 0.05$ ). In contrast, stalks, which were composed largely of internodes with a high content of highly lignified xylem vessels,<sup>9</sup> had the highest acid insoluble lignin and total lignin content. This result is also supported by the fact that the highest syringyl/guaiacyl ratio (S/G ratio) was observed for stalks (Table 2). As the syringyl lignin generated in the secondary cell wall increases, stalks, which serve as a backbone, become more rigid and provide better support for the entire

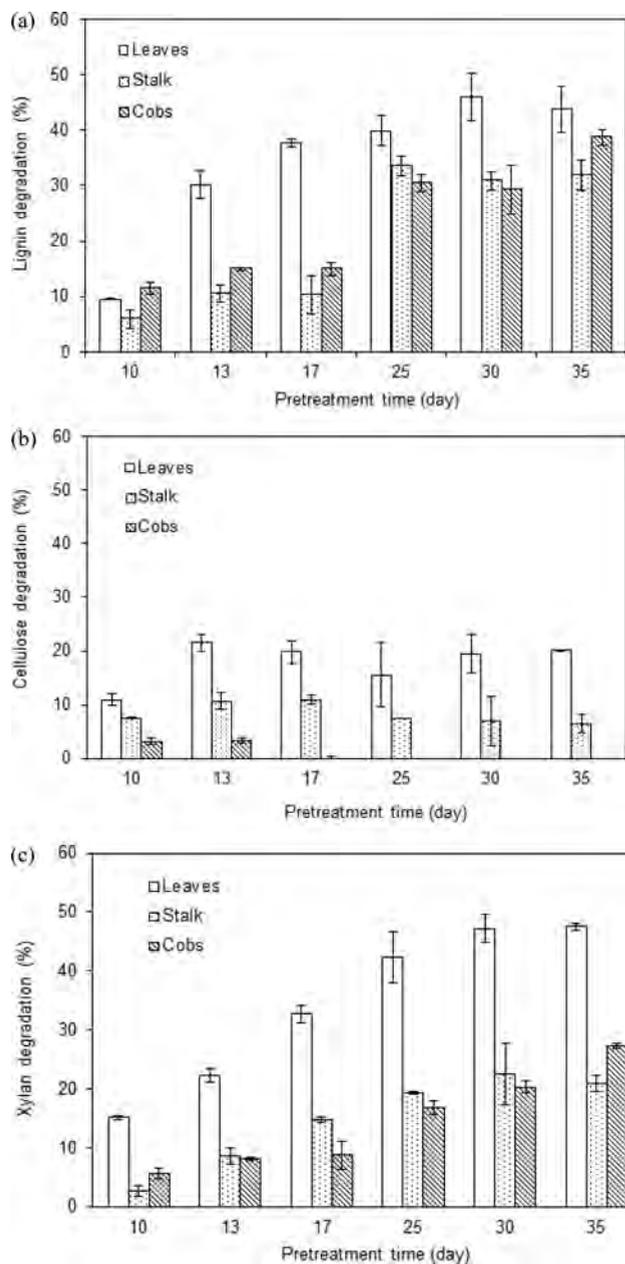
**Table 2. Changes of Lignin S/G Ratio in Fractions of Corn Stover after Pretreatment<sup>a</sup>**

parameter	time (day)	leaves	stalks	cobs
lignin content (%)	0	18.4 $\pm$ 0.5	23.0 $\pm$ 0.2	18.5 $\pm$ 0.5
	35	13.5 $\pm$ 1.0	18.2 $\pm$ 0.7	13.4 $\pm$ 0.3
lignin S/G ratio	0	0.6 $\pm$ 0.0	0.7 $\pm$ 0.0	0.4 $\pm$ 0.0
	35	0.5 $\pm$ 0.0	0.7 $\pm$ 0.0	0.4 $\pm$ 0.0

<sup>a</sup>The numbers after “ $\pm$ ” are standard errors for each mean.

plant.<sup>21</sup> It was evident that extractives and ash content of the three fractions were also different from one another. Leaves had high contents of extractives and ash compared with the other fractions, which was consistent with prior studies on corn stover and other plants.<sup>8,22</sup>

**3.2. Degradation.** The lignin degradation profile of each corn stover fraction is shown in Figure 1a. Lignin in leaves



**Figure 1.** Degradation of fractions of corn stover by *C. subvermisporea*: (a) lignin degradation, (b) cellulose degradation, and (c) xylan degradation.

tended to be degraded quickly by *C. subvermisporea* to a greater extent than that in stalks and cobs ( $p < 0.05$ ). For example, more than 30% lignin was degraded in leaves on day 13 while less than 15% lignin was degraded in stalks and cobs. Lignin removal in leaves was rapid in the first 17 days and then slowed down. In contrast, lignin in cobs and stalks was degraded slowly during the first 17 days followed by a rapid increase.

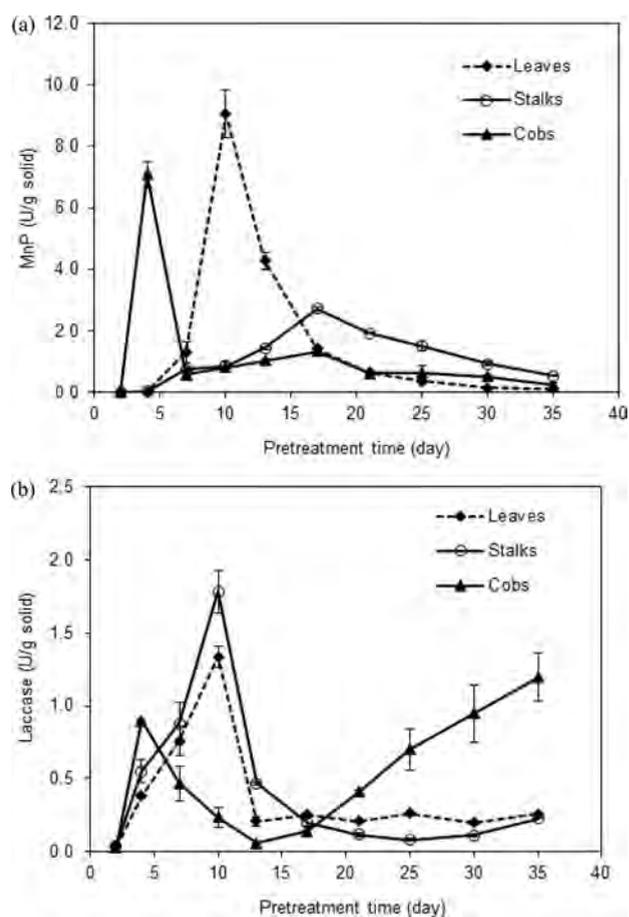
Apparently, the lignin content in corn stover fractions is not quite correlated to the degree of recalcitrance. For example, leaves had lignin contents very close to cobs but appeared to be the least recalcitrant fraction against the fungus, which suggested that factors (e.g., lignin composition, structure, and cross-linking) other than lignin content contributed to the resistance to fungal attack.

For lignin monomeric composition, a lower S/G ratio did not necessarily lead to more lignin oxidation by the fungus. Instead, the white rot fungus degraded syringyl lignin and guaiacyl lignin in all corn stover fractions almost to the same degree, thus resulting in no or only slight changes in S/G ratios after fungal pretreatment (Table 2).

Cellulose degradation caused by *C. subvermisporea* is shown in Figure 1b. Leaves had the highest cellulose loss (up to 20%) while cellulose loss for the other two fractions was below 10%. Unlike cellulose degradation, xylan degradation of corn stover fractions followed a similar degradation pattern to that of lignin (Figure 1c). Xylan in leaves was degraded more than in stalks and cobs ( $p < 0.05$ ) while the latter two had a similar rate of degradation ( $p > 0.05$ ). At the late stage of pretreatment, xylan degradation of all fractions tended to level off. Based on degradation results of corn stover fractions, it can be concluded that degradation of cell wall components was higher for leaves than for stalks and cobs, which was consistent with other findings.<sup>9,23</sup>

**3.3. Ligninocellulolytic Enzymes.** **3.3.1. Ligninolytic Enzymes.** To understand how *C. subvermisporea* acted on different fractions of corn stover, ligninolytic enzymes, mainly lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, secreted by this fungus, were investigated throughout the pretreatment process. Although LiP is a ligninolytic enzyme secreted by some white rot fungi, it was not detected during pretreatment of any corn stover fraction. This result is consistent with previous studies that found LiP was not secreted by *C. subvermisporea*.<sup>6,24</sup> MnP activity on leaves and cobs peaked on day 3 with a value of 7.09 U/g solid and on day 10 with a value of 9.04 U/g solid, respectively, and then decreased sharply (Figure 2a). In contrast, fungal cultures on stalks had a significantly lower MnP peak activity ( $p < 0.05$ ) during the first 13 days; however, on day 17, MnP was detected at a level of 2.72 U/g solid on stalks, which was significantly higher than that on the other two fractions ( $p < 0.05$ ). For laccase production, stalks and leaves had similar production profiles (Figure 2b). Laccase activity peaked on day 10, followed by a sharp decrease, and then leveled off. Cobs had a different laccase production profile from stalks and leaves. Laccase activity on cobs peaked on day 3 and then kept decreasing until day 13. Thereafter, laccase activity gradually increased and reached the highest level at the end of pretreatment.

The above results showed that expression of ligninolytic enzymes was feedstock-dependent, which is in agreement with other findings. Vicentim et al.<sup>25</sup> and de Souza-Cruz et al.<sup>26</sup> reported that MnP was the dominant oxidative enzyme in woody biomass cultured with *C. subvermisporea*. However, oxidative enzymes secreted by this fungus in sugar cane bagasse were different. In the present study, MnP appeared to be the dominant oxidative enzyme for all fractions except during the late stage for cobs. Although laccase was detected, it had a much lower ligninolytic activity than MnP, suggesting that both enzymes could work synergistically for initiating lignin oxidation. On the other hand, nutritional sources, inducers,



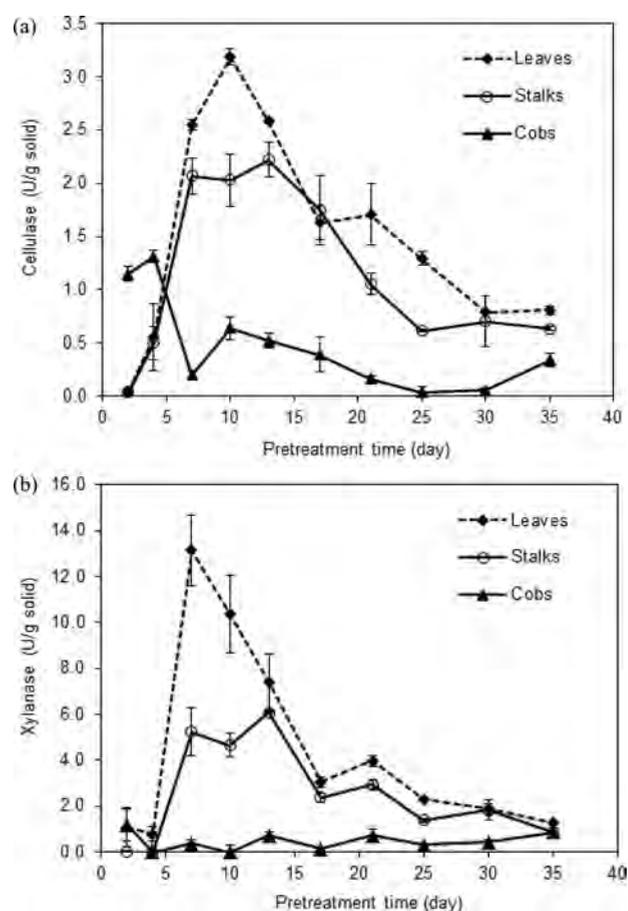
**Figure 2.** Production of ligninolytic enzymes during 35-d pretreatment of fractions of corn stover by *C. subvermispora*: (a) manganese peroxidase (MnP) and (b) laccase.

and radicals available from the plant biomass during decomposition generally regulate oxidative enzyme secretion.<sup>27,28</sup> Thus, corn stover fractions differing in composition and digestibility most likely contributed to different profiles of enzyme production of the fungus by differentially regulating ligninolytic enzyme expression.

Although enzyme production profiles varied among corn stover fractions or changed during the pretreatment for a single fraction, there is no direct relationship between levels of enzyme activity and the extent of lignin degradation. Prior studies have also shown ligninolytic enzymes did not influence lignin degradation directly.<sup>6,29,33</sup> For example, during solid state fermentation of *Eucalyptus grandis* by *C. subvermispora*, high levels of enzyme activity were detected after 15 days of decay but did not correlate with extensive lignin degradation.<sup>33</sup> However, due to the complicated and heterogeneous nature of biomass feedstocks, it is not clear how ligninolytic enzymes are involved during the early stage decay of biomass feedstocks. It is apparent that large molecular ligninolytic enzymes have difficulty in penetrating an unaltered plant cell wall due to its low permeability at the incipient stages. Low molecular mass agents, such as radicals and oxalic acids, have been reported to initiate biomass decay and facilitate the penetration of lignin-degrading enzymes.<sup>27,30</sup> Several studies demonstrated that *C. subvermispora* produced a variety of low molecular but highly active compounds including glyoxylic acid,<sup>31</sup> oxalic acid,<sup>30,31</sup> and unsaturated fatty acids<sup>32</sup> in liquid or solid state cultures.

Radical agents (e.g., peroxy and acyl radicals) were also generated from MnP-dependent peroxidation of this fungus while radical generation was dependent on the choice of culture conditions and substrates.<sup>27,31</sup> Thus, a series of oxidative reactions involved lignin depolymerization and eventually led to lignin mineralization. Although no correlation exists between enzyme production and lignin degradation, the fungus acted more vigorously on leaves than on the other two fractions, corresponding to more lignin removal.

**3.3.2. Hydrolytic Enzymes.** During fungal degradation, white rot fungi secrete hydrolytic enzymes to depolymerize polysaccharides into simple sugars for self-growth and metabolism. As a selective white rot fungus, *C. subvermispora* mainly secretes hemicellulolytic enzymes and assimilates hemicellulose-derived sugars as the main carbon source. Therefore, xylanase is detected at a high activity in fractions of corn stover cultured with this fungus (Figure 3b). Unlike leaves and stalks,



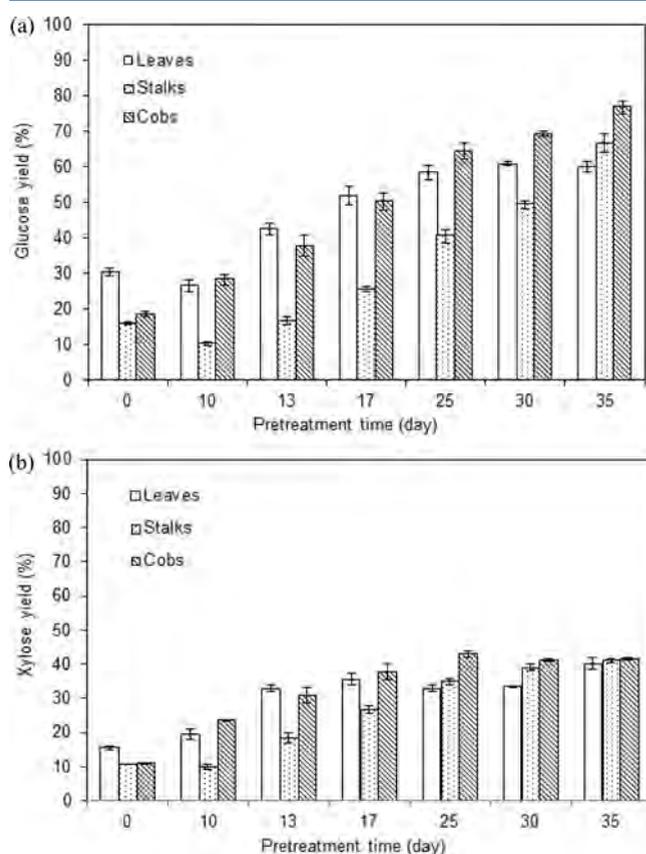
**Figure 3.** Production of hydrolytic enzymes during 35 d pretreatment of fractions of corn stover by *C. subvermispora*: (a) CMCase and (b) xylanase.

xylanase activity in cobs was unexpectedly low throughout the fungal pretreatment while there was substantial xylan degradation observed with this fraction. There may be a different type of xylanase secreted by the fungus in cobs, which was not detected by the assay method used in this study (i.e., birchwood xylan as the substrate). More efforts are needed to identify the xylanase produced in cobs by this fungus.

*C. subvermispora* is also known to lack a complete cellulolytic enzyme complex and, thus, is capable of preserving most of the

cellulose during fungal decay of biomass feedstocks. For woody biomass, only up to 7.3% glucan loss was detected from *C. subvermispora* and cellulose activity was almost undetectable.<sup>29,33</sup> However, cellulase activity, revealed as CMC activity, was evident in corn stover fractions, especially with leaves and stalks (Figure 3a), which corresponded to significant cellulose loss in these two fractions (Figure 1b). This finding was not consistent with our previous study,<sup>6</sup> most likely due to variations in cellulolytic activity of the fungus associated with different corn stover fractions. For example, the more digestible the leaves were, the more active hydrolytic enzymes of the fungus were. Nevertheless, xylanase was still the dominant hydrolytic enzyme for this selective fungus, irrespective of the corn stover fraction; hence, a large amount of xylan was consumed throughout the pretreatment.

**3.4. Enzymatic Hydrolysis.** Lignin plays a critical role in biomass recalcitrance to cellulolytic enzymes. It is generally perceived that low lignin content results in high cellulose digestibility. However, as seen in Figure 4a, untreated leaves



**Figure 4.** Sugar yields of fractions of corn stover pretreated by *C. subvermispora*: (a) glucose yield and (b) xylose yield.

had 31% glucose yield, which was significantly higher than cobs ( $p < 0.05$ ), although the leaves had similar lignin content to cobs. In contrast, the glucose yield of cobs was similar to that of stalks ( $p > 0.05$ ) although both fractions had different lignin content. The fact that no correlation existed between lignin content and cellulose digestibility across the fractions suggests factors other than lignin content may affect sugar release. Factors, such as lignin structure and lignin subunits, have been shown to strongly influence cellulose digestibility.<sup>4</sup> Recent studies, which focused on the effects of lignin monomeric

composition on sugar release, indicated that the lignin S/G ratio generally has a positive relationship to sugar release of pretreated samples.<sup>34,35</sup> For untreated biomass feedstocks, Studer et al.<sup>35</sup> demonstrated that lignin content below 20% negatively affected sugar release of natural *Populus*, irrespective of the lignin S/G ratio. Our observation did not agree with the above findings as no clear trend was found between the lignin S/G ratio and sugar release.

For untreated plant fractions, it is well documented that plant leaves are more easily hydrolyzed than stems. When pretreatment was applied, plant leaves generally required less severe pretreatment conditions and resulted in a high conversion rate. Duguid et al.<sup>11</sup> showed that wheat straw leaves, as compared to stem internodes and nodes, gave more sugar release after very mild pretreatment. Switchgrass leaves also had higher digestibility than stems.<sup>22</sup> Results from this study also showed that high glucose yield from untreated or mildly pretreated leaves can potentially lower biorefining-associated production costs.

Fungal pretreatment facilitated enzymatic hydrolysis of corn stover fractions. Glucose yields of leaves increased with pretreatment time and then leveled off after 30 days. In contrast, glucose yields of stalks and cobs increased steadily over pretreatment time. For 25 days of pretreatment, cobs appeared to have similar glucose yields to leaves ( $p > 0.05$ ) but achieved significantly higher glucose yields than stalks ( $p < 0.05$ ). About 78% glucose yield was obtained from cobs pretreated for 35 days, which was about 60% higher than that of untreated cobs. Stalks also had markedly improved glucose yields over the untreated, resulting in a 67% glucose yield after 35 days of pretreatment. For each fraction, cellulose digestibility was closely related to the lignin degradation as the pretreated residues were much less resistant to enzymatic hydrolysis due to lignin removal. A relatively linear, positive relationship ( $R^2 = 0.90\text{--}0.97$ ) was also found between cellulose digestibility of corn stover fractions and lignin degradation but was negative between cellulose digestibility and remaining lignin residue in pretreated solids. This also indicated that lignin residue after fungal pretreatment became the dominant factor affecting sugar release, which was in agreement with our previous study on whole corn stover.<sup>6</sup> For a single fraction, as the lignin content in the pretreated material decreased, the sugar release increased. Thus, the performance of the fungus on lignin oxidation strongly affected the extent of lignin removal and subsequent sugar yield. Although lignin degradation for stalks and cobs was not as high as for leaves, its effect on enzymatic hydrolysis of stalks and cobs was more pronounced than on leaves. As indicated in Figure 4a, fungal pretreatment increased the glucose yield by up to 30% for leaves and by more than 50% for stalks and cobs.

Xylose yields of all fractions pretreated over time are shown in Figure 4b, which followed a similar pattern to glucose yields except during the later stage of pretreatment. Xylose yields of about 35% were observed for all fractions after 35 days of pretreatment. As mentioned previously, leaves had more readily digestible sugars, and thus also gave a xylose yield of 15.5% from untreated leaves. A comparison between stalks and cobs indicates that the former had a significantly lower xylan digestibility ( $p < 0.05$ ) during the first 25 days of pretreatment, which could be attributed to a higher degree of substitution (e.g., acetyl groups, ferulic acids) in the xylan backbone of stalks. Recent work by Van Dongen et al.<sup>36</sup> has demonstrated that xylan in corn stover, mainly composed of stalk xylan, is

heavily interlinked to lignin and arabinofuranose via feruloylation while this complexity is not as evident for cob xylan. With extended pretreatment time, it appeared that the fungus broke down more of the xylan backbone as well as its side chains. These results also indicate that fungal pretreatment is more effective when dealing with more recalcitrant fractions.

**3.5. Comparison of Glucose Production between Corn Stover Fractions and the Whole Plant.** Glucose production from fractions of corn stover and the whole plant is compared in Table 3. It should be noted that calculated

**Table 3. Comparison of Glucose Production between Fractions of Corn Stover and the Whole Plant**

parameters	leaves	stalks	cobs	total	whole plant <sup>a</sup>
cellulose (% of each feedstock)	33.8	40.7	34.1		38.6
weigh distribution <sup>b</sup> (% of the whole stover)	16.0	43.0	41.0		100.0
theoretical glucose production (kg per ton stover)	60.1	194.5	155.3	409.9	427.6
glucose yield of the untreated (%)	30.5	15.9	18.5		22.0
maximum glucose yield of the pretreated (%)	60.0	66.6	76.8		66.3
maximum glucose production of the untreated (kg per ton stover)	18.3	30.9	28.8	78.0	94.1
maximum glucose production of the pretreated (kg per ton stover)	36.0	129.5	119.2	284.7	283.6
glucose production of the untreated (kg per ton fraction)	114.4	71.9	70.2		94.1
maximum glucose production of the pretreated (kg per ton fraction)	225.2	301.1	290.8		283.6

<sup>a</sup>Data from our previous study.<sup>6</sup> <sup>b</sup>Data from Montross and Crofcheck.<sup>23</sup>

theoretical glucose production as a sum of three fractions is about 5% lower than that from the whole plant, mostly likely due to errors associated with cellulose determination and fraction distribution. After 35 days of pretreatment, maximal glucose production of the three fractions was close to that from the whole plant. Although fractionating corn stover did not result in a higher total glucose production, fungal pretreatment can be optimized in order to save operational costs while maximizing sugar release. For example, for leaves, a less severe pretreatment can be applied; in this case, 25 days were actually long enough for obtaining a maximal glucose yield. On the other hand, on a per ton of feedstock basis, untreated leaves gave 114.4 kg total glucose, about 22% higher than the whole plant. After fungal pretreatment, cobs and stalks were more attractive than leaves and the whole plant in terms of maximal glucose production. Therefore, when both soil sustainability and sugar conversion efficiency are considered, harvesting only cobs and stalks could be the most desirable alternative since leaves could be easily broken down in the field. Moreover, leaves have high ash contents (Table 1), which is desirable for soil erosion control.<sup>11</sup> However, economic analysis is required to evaluate selective harvesting for a biorefinery based on fungal pretreatment.

#### 4. CONCLUSION

In this study, the effectiveness of fungal pretreatment by *C. subvermispora* on different corn stover fractions (leaves, stalks,

and cobs) was evaluated by enzymatic hydrolysis. Leaves appeared to be the least recalcitrant fraction to the fungus as the most vigorous degradation was observed with this fraction. However, the sugar yield of leaves was less significantly improved by fungal pretreatment. Among the three fractions, cobs had the highest sugar yields as a result of fungal pretreatment but required a longer pretreatment time. Our findings indicated that selectively processing corn stover fractions based on fungal pretreatment could be beneficial to sugar recovery.

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##### Notes

The authors declare no competing financial interest.

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