The Xylan Delignification Process for Biomass Conversion to Ethanol

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Summary
An extrusion process melded with alkaline peroxide chemical pretreatments allows the lignin and hemicellulose in biomass to be solubilized, and the cellulose component to be made available for enzymatic breakdown. This process is called the Xylan Delignification Process (XDP). In this paper, some results of the XDP on promoting enzymatic breakdown and SSF of corn stalks, switch grass and straw are reported. It was found that the XDP process allowed quick (6 hour) and reasonably complete (85-88%) hydrolysis of the cellulose fraction of corn stalks, but was less effective in allowing utilization of the switch grass with 76% yield noted in 24 hours. Solubilization of the lignin and hemicellulose were not achieved on a first set of corn stalk, switch grass, and straw samples, but was noted on a second straw sample.

Key Words: Biomass Pretreatment, Ethanol, Saccharification, SSF, Delignification

Introduction
Biomass is a mix of three basic components, lignin, cellulose and hemicellulose. Lignin serves as a sort of 'glue' giving the biomass fibers its structural strength, while hemicellulose and cellulose polymers are the basic building blocks of the fibers. In order to break down the hemicellulose and cellulose to sugars, the basic structure of the biomass must be attacked. Once the structure of the biomass is disrupted, the hemicellulose and cellulose can be converted to sugars enzymatically. The conversion of biomass (through enzymatic techniques) to ethanol consists of four basic steps, 1- pretreatment of the biomass to allow enzymatic attack of the bio-polymers, 2- conversion and fermentation of the hemicellulose/xylose fraction, 3- conversion and fermentation of the cellulose/glucose fraction, and 4- separation and concentration of the ethanol. There are many different technologies currently under development to convert biomass to ethanol, but despite some rather expensive development efforts, there are no successfully operating biomass to ethanol plants running presently in the US to our knowledge.
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Pre-treatment

In this work we utilized the peroxide extrusion process of Xylan. The Xylan-Delignification-Process (XDP) utilizes extrusion technology in conjunction with alkali soak and hydrogen peroxide injection. Carr and Doane (1) reported on the use of a similar process for straw pretreatment using NaOH and anthraquinone, and Chen and Wayman (2) also utilized this sort of treatment for Aspen wood chips adding SO2 prior to extrusion with a 4% hot NaOH soak post-extrusion delignification. The Xylan method continuously treats lignocellulosic biomass by first reacting the biomass with a medium containing an aqueous solution of alkali agent (pH 11.5) which softens the lignin and allows water to enter the biomass. The cellulosic biomass is then fed into a pressurized extruder/reactor in an oxygen atmosphere at a temperature between 235-275 °F and pressures up to 400 psi. These high temperatures and pressures allow minimization of chemicals as compared to other technologies for cellulose pretreatment (low acid-high temp, or high acid-low temp). The mechanical extrusion system mixes, grinds, sterilizes, and disrupts the cellulosic biomass cell walls. Exiting the reactor barrel is a liquid/solid mixture stream containing lignin and hemicellulose sugars, and cellulose fibers, suitable for paper, cattle feed, or enzymatic hydrolysis to glucose.

Figure 1. Xylan Delignification Process

Hydrogen peroxide is added to the barrel of the extruder to help catalyze the breakdown of the fibrous biomass structure. The wet/fibrous product from the extrusion process consists of a lignin/soluble hemicellulose stream and a fibrous cellulose. Squeezing and washing the cellulose gives two streams, the lignin/soluble hemicellulose, and the solid fibrous cellulose. The cellulose stream can be used for ethanol production, paper production, or cattle feed.
The liquid lignin/soluble hemicellulose stream is easily converted to xylose enzymatically, after which it can be converted to ethanol using various fermentation strategies. In previous lab tests, when treating this stream (from a pre-treated wood chip process) with hemicellulase, good conversion of the hemicellulose to xylose was noted, with 20 g/l xylose being determined after hydrolysis along with 2.2 g/l of glucose (3). The cellulosic fibers stream can then be broken down to glucose via a similar enzymatic treatment. The cellulosic fiber stream, when added to water and hydrolyzed with cellulase gave a 10.7 g/l ethanol product during a Simultaneous Saccharification Fermentation (SSF) with a 31.9% yield of ethanol from dry matter yield (3). Co-products of the fermentation included lactic acid and acetic acid at 3.3 and 1.6 g/l final concentrations respectively with 4.2 g/l glucose also remaining unfermented.

Methods
This project was funded largely by NREL, who have established a set of standard procedures to allow work by various contractors to be directly comparable (4). These procedures were then used for determinations of biomass compositions and fermentation broths as described below.

Ethanol- 0.1 ml. sample is diluted with 0.9 ml of solution containing isopropyl alcohol (internal standard) and analyzed using a gas chromatograph fitted with a carbowax column.

Total solids of biomass- The sample (1-5 g) is held at 105 C for between 3 to 24 hours - until constant weight is attained.

Klason Lignin- The sample is digested in 72% sulfuric acid, with the lignin remaining undigested and determined by gravimetric analysis. Three samples were run to establish the repeatability of the analysis.

Acid soluble lignin-Acid soluble lignin is determined by spectrophotometric absorbance at 205 nm on the filtrate.

Ash- The sample is ashed in a furnace at 575 C for 3-5 hours. There may be a slight increase in ash due to the chemicals added in the XDP processing.
Cellulase Activities - Cellulase, Lot # 17-92262-09 from Env. Biotech. Inc. was utilized to release sugars from the biomass. The FPU (enzyme quantity able to breakdown 4% of a filter paper solution) of the cellulase enzymes provided was determined as 67.9 FPU/ml av. dev +/- 0.76 FPU/ml with three determinations run.

Saccharification of biomass samples - The hydrolysis of the biomass with the cellulase was measured by taking 0.1 g of cellulose in the biomass sample (by calculation based on dry weight and cellulose composition) added to 9.9 ml water. 66.7 microliters of cellulase enzyme is then added to the solution, and the sample is then allowed to digest at 50°C. Glucose liberation rate is then measured.

SSF of XDP samples - Approximately 30 g/L of cellulose (based on the cellulose content of the biomass analysis) was simultaneously saccharified and fermented with an enzyme dosage of 25 FPU/ml in the fermentation mixture (actual biomass dosages are given in Table 1). Yeast S. cerevisiae D5A was used unless noted otherwise with the fermentation carried out at 38°C. The biomass stream was fermented with three fermentations carried out simultaneously. An alpha-cellulose sample was fermented as a baseline control sample. Table 1 gives the amount of biomass (wet basis) added to the various saccharification and fermentation trials with total fermentation broth being 50 ml.

Table 1.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Figure</th>
<th>Substrate</th>
<th>g Biomass (wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>008-Hydr</td>
<td>1</td>
<td>XDP corn stalks</td>
<td>2.6g</td>
</tr>
<tr>
<td>008-Hydr</td>
<td>2</td>
<td>XDP switch grass</td>
<td>2.7g</td>
</tr>
<tr>
<td>008-Hydr</td>
<td>3</td>
<td>fresh corn stalk</td>
<td>2.0g</td>
</tr>
<tr>
<td>008-SSF</td>
<td>4</td>
<td>XDP corn stalk</td>
<td>7.0g</td>
</tr>
<tr>
<td>008-SSF</td>
<td>5</td>
<td>XDP switch grass</td>
<td>4.5g</td>
</tr>
<tr>
<td>008-SSF</td>
<td>7</td>
<td>XDP corn stalk</td>
<td>5.0g</td>
</tr>
</tbody>
</table>

Pretreatment - The process begins with the addition of sodium hydroxide, at approximately 3# of 50% NaOH solution per 100 # of biomass. The base was mixed with the shredded biomass in a 15HP Grainger stainless steel mixer for a period of fifteen minutes. The biomass was then taken to a "cram feeder" attachment to a SX 20 Wenger extruder. The barrel of the extruder was heated to 200°C using low pressure steam. Hydrogen peroxide, at about a 9% solution strength, was injected near the entrance to the extruder barrel at a rate of about 1 # per 100# of biomass. A pressure of about 300 to 400 psi
develops within the extruder barrel. After about a 15 second residence time, the biomass 'exploded' from the die at the end of the extruder barrel. A 3/8" die aperture was used when processing the straw, corn stalks, and switch grass used in this project.

Lab Analysis

The XDP treated samples of straw, corn stalks and switch grass were received at Purdue from Mr. Tyson of Xylan, Inc. Biomass samples were sealed in zip-lock bags and frozen to prevent deterioration. The samples were tested for basic composition as given below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Corn Stalk</th>
<th>Switch Grass</th>
<th>Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total Solids</td>
<td>38.0%</td>
<td>56.8%</td>
<td>87.9%</td>
</tr>
<tr>
<td>av. dev.</td>
<td>+/- 0.47</td>
<td>0.85</td>
<td>0.3%</td>
</tr>
<tr>
<td>2. Ash</td>
<td>15.0%</td>
<td>11.4%</td>
<td>10.7%</td>
</tr>
<tr>
<td>3. K. Lignin</td>
<td>18.8%</td>
<td>21.0%</td>
<td>23.4%</td>
</tr>
<tr>
<td>4. A. S. Lignin</td>
<td>2.0%</td>
<td>1.7%</td>
<td>0.3%</td>
</tr>
<tr>
<td>5. Acid Hydrol.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Glucose</td>
<td>39.2%</td>
<td>31.4%</td>
<td>39.5%</td>
</tr>
<tr>
<td>av. dev.</td>
<td>+/-0.34</td>
<td>1.1</td>
<td>1.0%</td>
</tr>
<tr>
<td>b) Xylose</td>
<td>24.9%</td>
<td>20.5%</td>
<td>19.1%</td>
</tr>
<tr>
<td>av. dev.</td>
<td>+/-0.3</td>
<td>2.6</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

Results

Enzymatic Hydrolysis of XDP Samples  The results of enzyme hydrolysis (w/ fermentation nutrients) of XDP treated corn stalks are shown in Figure 1. The yield or conversion efficiency (as defined as grams cellobiose and glucose released per gram of cellulose) can be seen to be about 85% within 7 hours. During hours 7 through 24, cellobiose concentration drops with a concomitant increase in glucose concentration. Final glucose concentrations of 4.8 g/l glucose, 3.2 g/l cellobiose, and 2.6 g/l xylose were noted. The variability between the three replicates is given as a range about the average.

Similar data for XDP treated switch grass is shown in Figure 2. Conversion efficiencies of 78% were noted at 24 hours, with conversion being somewhat slower than was seen with the XDP treated corn stalks, although a 65% conversion yield was noted at 7 hours. Cellobiose levels were not observed to drop, although glucose levels continued to rise slowly from hours 7 to 24 of the treatment. As a control, alpha-cellulose showed a 97% conversion to cellobiose and glucose in a 24 hour period.
The effectiveness of the XDP treatment as compared to untreated corn stalks was determined finally as a test of the XDP efficacy. 'Fresh' corn stalks were gathered from an Indiana field about 2 months after harvest (late October '94), manually cut into short 2 cm lengths, milled in Waring blender, and sieved using a 40 mesh sieve. This untreated corn stalk sample was then subjected to the same enzymatic evaluation as the pretreated samples. As shown in Figure 3, conversion was considerably slower, with only a 50% yield noted in 7 hours, and a final yield of 72% noted at 24 hours.

It was determined that the yeast nutrients had a beneficial effect or possibly some cellulasic enzymatic properties when the hydrolysis experiments were repeated without the fermentation nutrients on the XDP corn stalks, the XDP switch grass, and the alpha cellulose. XDP treated corn stalks showed a much slower release of sugars, with a 65% yield determined at 95 hours, and a final yield of 75% determined at 170 hours. Switch grass showed similar drop in performance without the fermentation nutrients a 55% yield was determined at 50 hours, with a final yield of 72% noted at 170 hours (5). Alpha cellulose as well was found to be much slower to convert with a 98% conversion recorded at 160 hours as compared to 24 hours when nutrients were present.

SSF of XDP treated Biomass The Simultaneous Saccharification and Fermentation (SSF) of the biomass samples was next tested with the D5A S. cerevisae as provided by NREL, and then repeated using some of our labs yeast cultures. The SSF of XDP treated corn stalks is shown in Figure 4. We measured a 46% yield in 48 hours with an ethanol concentration of 12 g/liter, after which time there was little change in ethanol level over the next 75 hours. SSF of the XDP treated switch grass is shown in Figure 5 with a 33% yield noted at 48 hours, after which time ethanol levels were observed to drop with time. An ethanol concentration of close to 6 g/l was reached at 48 hours. SSF of alpha cellulose was run as a control with 16.5 g/l ethanol reached at 100 hours. Yields from the various biomass samples are compared in Figure 6, corn stalks and alpha cellulose fermentation rates were comparable over the first 50 hours, after which alpha cellulose continued to convert, while the corn stalk fermentation was stagnant. Switch grass performance was significantly lower than corn stalks.

SSF of the XDP treated cornstalks was run using a flocculent S. cerevisae maintained in our labs, strain NRRL 11878. This yeast is not as temperature tolerant as the D5A strain, so the fermentation was run at 30°C. Performance was much worse at this lower temperature, with only a 25% yield noted at 48 hours. A residual glucose
concentration of 2 g/l was noted with no measurable xylose observed. Co-culturing of glucose fermenting (S. cerevisae 11878) and xylose fermenting (P. stipitus NRRL 11545) yeast strains was next tested (similar to studies by Grooten et al, (6,7)). Yield improved slightly to 33% in about 48 hours, with lower levels of glucose determined (0.5 g/l) at 70 hours. Again, no xylose was noted in our analyses.

A comparison of the SSF experiments on XDP corn stalks is shown in Figure 7. The higher temperature SSF gave the best yields. The use of even higher temperature yeast, perhaps K marxianus strains might be explored in the future (8). In results not shown here, it was found that contamination of the SSF broth led to poor fermentation performance. We found it important to filter sterilize the cellulase enzyme to prevent any contamination of the SSF experiments.

**Solubilization of lignin and hemicellulose by the XDP**

The XDP treatment of biomass should have the effect of solubilizing the lignin and hemicellulose fractions of the biomass. The effectiveness of the XDP treatment was evaluated by a few tests in which the biomass was rinsed with hot water to release the soluble hemicellulose and lignin. If the XDP were totally effective, little lignin or hemicellulose would remain after the rinsing, with the fiber being largely cellulose. Acid hydrolysis of the rinsed remaining cellulose fiber should release only glucose. Solubilization tests of four substrates are shown in Table 2. As per this table, only 12.2-15.6% of the solids obtained during '94 were soluble, but the second straw sample received in early 1995, which had been run through a different extruder, showed good solubility (Straw II).

<table>
<thead>
<tr>
<th></th>
<th><strong>Corn Stalks</strong></th>
<th><strong>Switch Grass</strong></th>
<th><strong>Wheat Straw</strong></th>
<th><strong>Straw II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Solid/50 g</td>
<td>20.5</td>
<td>28.3</td>
<td>44.1</td>
<td>44.9</td>
</tr>
<tr>
<td>Soluble Solids</td>
<td>2.52</td>
<td>4.4</td>
<td>6.6</td>
<td>14.5</td>
</tr>
<tr>
<td>% Soluble Solids</td>
<td>12.20%</td>
<td>15.60%</td>
<td>14.90%</td>
<td>32.30%</td>
</tr>
</tbody>
</table>

The enzymatic hydrolysis of this second sample of straw (Straw II) was tested. The product was separated into two streams as per Figure 1, a solids fraction remaining after water extraction, and the liquid stream containing the solubles. The liquid stream was treated with hemicellulase as shown in Figure 8, with an initial xylose concentration of 5.3 g/l increasing to a final concentration of 6.8 g/l in 3 hours. Similarly, the solid stream was hydrolyzed as shown in Figure 9. We measured a 68% yield of the cellulose to sugars
(yield defined based on compositions shown in Table 1) in 12 hours with a fairly large amount of xylose also released (an initial concentration of 2 g/l increasing to 8.0 g/l over 12 hours) indicating that the recovery of hemicellulose/xylose in the liquid extraction was not total. A mass balance on the xylose indicates 48% of the hemicellulose was extracted in the hot water extraction, perhaps more could have been extracted in repeated washings. These results make us suspect that the correct extrusion temperatures/pressures were not quite reached during the pretreatment process in the original 3 samples, but was achieved in the second straw sample (Straw II).

Discussion

We feel that the XDP for biomass pretreatment could be coupled with a two stage fermentation process to produce ethanol from biomass in an efficient and economical fashion. The solubilized liquid stream could be fermented using an efficient xylose to ethanol yeast, while the cellulose could be fermented using either a thermotolerant glucose to ethanol yeast/bacteria in an SSF type reactor, or pre-hydrolysed and fermented. Co-fermentation of xylose and glucose is difficult as noted by Grooten et al. (6,7), although apparently some progress is being made on the development of a genetically altered yeast which can sequentially ferment both xylose and glucose (9). To achieve a high concentration of xylose, a counter current washing of the biomass could be used. In a preliminary simulation of this process, we found that when a solution was used 4 times to extract solubles from four straw II samples, acetic acid built up in an almost linear fashion from 1.2 g/l in the first rinse to 7.6 g/l in the fourth rinse. A recent study (10) suggests that the acetic acid is quite inhibitory to P. stipitus, with no fermentation noted at 5 g/l acetic acid and little at 2 g/l. The removal of the acetic acid was required to achieve a fast and efficient conversion of the hemicellulose hydrolysis.

Acknowledgments

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References


Figure 1. Hydrolysis of XDP-treated Corn Stalk
Figure 2. Hydrolysis of Switch Grass

![Graph showing the hydrolysis of switch grass over time, with content and yield as variables.](image-url)
Figure 3. Hydrolysis of Non-treated Corn stalk

The graph shows the hydrolysis of non-treated corn stalk over time (hour) in terms of content (g/l) and yield (%). The graph includes lines for celbio, glucose, xylose, and yield, each represented by different markers. The x-axis represents time in hours, ranging from 0 to 25, while the y-axis represents content in g/l and yield in %, ranging from 0 to 3 for content and 0 to 80 for yield. The data points are plotted with error bars indicating variability.
Figure 4. SSF of XDP-treated Corn Stalk
Figure 5. SSF of XDP-treated Switch Grass

- Ethanol → Yield

(Yield) (%)

Time (hour)

Content (g/l)
Figure 6. Comparison of Biomass SSF

The graph compares the yield of different biomass sources over time. The x-axis represents time in hours, ranging from 0 to 125, and the y-axis represents yield percentage (%) ranging from 0 to 60.

The graph shows three different lines:
- XDP Corn Stalk
- XDP Switch Grass
- a-Cellulose

The lines indicate the increase in yield over time for each biomass source.
Figure 7. Comparison of Biomass SSF  
(Different Strains and Temperature)

![Graph showing the comparison of biomass SSF at different conditions.](image-url)
Figure 8. Hydrolysis of Extracted Liquid
(XDP-treated Wheat Straw II)

Content (g/L)

Time (hour)

- Cellulose
- Glucose
- Xylose
- Yield(Cellulose)
- Yield(Hemicellulose)
Figure 9. Hydrolysis of Extracted Wheat Straw Solids
(XDP-treated Wheat Straw II)

- Cellulose
- Glucose
- Xylose
- Yield (Cellulose)