Extraction of Hemicellulose Sugars from Wood Waste (from Construction and Demolition Activities) to Produce Ethanol for Fuel

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Final Report

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Background

Objectives
The objective of this research project was to evaluate the influence of ultrasonic stimulation and supercritical carbon dioxide treatment of wood waste from construction and demolition activities to provide high yields of pentose (e.g., xylose, \(\text{C}_5\text{H}_{10}\text{O}_5\)) and/or hexose sugars (e.g., glucose, \(\text{C}_6\text{H}_{12}\text{O}_6\)) that are easily fermentable to ethanol. The goal was to find conditions that result in efficient and high yield extraction of the hemicellulose sugars without the attendant degradation of sugars that produce materials inhibitory to subsequent fermentation, namely aromatic aldehydes like furfural (\(\text{C}_5\text{H}_4\text{O}_2\)) and/or 5-hydroxymethy-2-furfural (\(\text{C}_6\text{H}_6\text{O}_3\)). It has been previously demonstrated that liquid and supercritical carbon dioxide are capable of rapid penetration of materials. Additionally, sonication of carbon dioxide mediated reactions can result in significant increases in reaction rates. Addition of catalysts for conversion of hemicellulose to monomeric sugars could also enhance the rate of conversion and are part of the current investigation.

Project Description
It has been reported that 28.7% of the waste being landfilled in Wisconsin consists of construction and demolition (C&D) debris. This amounts to over 850,000 tons of solid waste per year.\(^1\) A significant amount of this C&D waste is untreated wood. In addition, there is considerable interest in developing processes for converting cellulosic materials to specialty chemicals, as well as energy. A national interest has been identified as conversion of cellulosics to fuel ethanol. Because ethanol can be blended with gasoline or used by itself as an automotive
fuel, there has been an expectation that large scale production of ethanol would reduce the
dependence of the United States on imported oil. Since cellulose is the most abundant renewable
biopolymer and is a major constituent of most plants, there is great interest in developing
processes to efficiently convert cellulosics to ethanol and chemicals. As long as the energy used
to convert cellulosics to fuel and chemicals comes from a renewable source, the conversion and
use of the derived fuel is greenhouse gas neutral as the carbon dioxide generated by the fuel use
of the ethanol will ultimately be recaptured by plants in the photosynthesis cycle that produces
cellulosics. Both the USDA and the US Department of Energy are funding major projects to
develop processes for converting cellulosics to ethanol.

Conversion of cellulosic materials to ethanol has been investigated for over one hundred and
fifty years. As early as 1819 it was determined that acid hydrolysis of cellulose could produce
sugar that could be fermented to alcohol. During and after World War I, numerous plants were
built in Europe to convert wood into ethanol for fuel. The use of wood as the starting material
has many attractive features. Unlike annual plants like switchgrass and corn stover, trees can be
left growing until there is a need to harvest them. With annual plants, they are typically harvested
once or twice a year. In the interim times the plants are maturing and there is no harvest. Trees
are also a relatively dense form of biomass that are already harvested on a large scale.

Although simple chemical and enzymatic methods exist to convert wood to fermentable sugars,
the overall efficiency of these processes offer significant room for improvement. Part of the
inefficiency results from the need to separate the non-carbohydrate fractions of the wood from
the carbohydrate portions. In most woody plants, the cellulose and hemicelluloses (the parts of
the plant that are used for ethanol production) are in a matrix with lignin. Lignin is a complex
polymer composed of a variety of phenyl propane units. While processes have been developed to
liberate the carbohydrate portions from the lignin, these usually result in some degradation of the
carbohydrates. The degradation products in many instances adversely impact the subsequent
fermentation of the carbohydrate fractions. Although processes have been developed to remove
these toxicants, the added processing and attendant losses inherent in the added steps reduce the
overall efficiency of conversion to ethanol.
Current processes for converting cellulosic residues involve a prehydrolysis step to selectively remove the hemicellulose from the matrix. Although this is a hydrolysis of the hemicellulose portions, it is termed prehydrolysis because it precedes the hydrolysis of the cellulosic portions of the substrate. The need to separate the hemicellulose sugars from the cellulose sugars is a consequence of the need to use different enzymes and organisms to ferment five carbon sugars (pentoses, from hemicelluloses) and six carbon sugars (from the cellulose portion) to ethanol.

The processes for removing the hemicellulose sugars generally involve treatment of the materials with acid. Mineral acids such as hydrochloric or sulfuric acid as well as organic acids such as acetic acid have been investigated. These processes require elevated temperatures (i.e., above 100°C) and extended reaction times to provide efficient removal of the hemicellulose sugars. The residues are heated with the dilute acid to effect hydrolysis and dissolution of the hemicelluloses. This is followed by separation and washing of the undissolved materials and neutralization of the hydrolysis liquors containing the hemicellulose sugars and oligomers. The prehydrolysis reaction is a kinetically controlled process that is influenced by the penetration rate of the acid into the fibrous structure and the release of the hydrolyzed sugars. This prehydrolysis step results in a dilute solution of pentose sugars and oligomers. Acid hydrolysis can result in the formation of furfural, furfuryl alcohol and acetic acid that are inhibitory to enzymatic conversion of the pentose sugars to ethanol. This project will focus on evaluating alternative strategies for extracting the hemicellulose, namely, supercritical fluid extraction with carbon dioxide, as well as sonication.

Supercritical carbon dioxide extraction of materials has a long commercial history in the extraction of natural products. One of the major processes for decaffeinating coffee involves the use of supercritical carbon dioxide. Because supercritical carbon dioxide is non-toxic, easily handled (supercritical conditions are 31°C and pressure of 74 bar) and readily available, its commercial use is becoming common for extraction and separation of materials. The intent of the investigation is not simply the extraction of materials in a conventional manner. The object of this proposal is to investigate the combination of supercritical carbon dioxide treatment with ultrasonic stimulation. Recent work\textsuperscript{2,3} has shown that sonication of materials in the presence of supercritical or near supercritical carbon dioxide can increase reactions rates by orders of
magnitude. It is believed that sonication enhances reactivity through cavitation, whereby the collapse of the bubbles caused by cavitation results in localized high temperatures and pressures.\(^4\) Of particular interest is the observation that sonication of mixtures of water and near critical carbon dioxide increased the hydrolysis rate of organic molecules up to 200 fold.\(^5\)

**Experimental Section**

**Wood Samples from Construction Lumber**

Two different types of wood were utilized in this study; a sample of softwood (construction lumber: S-P-F, 2 x 4 stud, KD Select, S-Dry) and a sample of hardwood (aspen, 1 x 2 Select) were both purchased commercially. The wood samples were transformed into sawdust using a circular saw. This sawdust was subjected to a variety of extraction treatments by adding acid and processing with supercritical carbon dioxide or sonication. Some of the specific variables that were examined in this study included:

- Acid Type (hydrochloric, sulfuric, and acetic)
- Acid Strength
- Reaction Time
- Reaction Temperature

After performing the reaction for a specified period of time, the wood was rinsed with water to remove any water soluble reaction products. In some cases the wood was filtered and the filtrate analyzed for the presence of any reaction products, and in other cases the water in contact with the wood was analyzed without filtration. A variety of analytical methods were employed to identify and quantify the products of the reaction including: a TOC (total organic carbon) analyzer, an HPLC (high performance liquid chromatograph), and a GC-MS (gas chromatograph with mass spectrometer detector). Further details are included under sections corresponding to each analytical technique. Finally, the remaining solid material was isolated, dried and weighed to determine the mass change due to the reaction.
Supercritical Fluid Carbon Dioxide Reactions

A Suprex Prepmaster supercritical fluid carbon dioxide instrument was used in this project. All reactions were performed at a pressure of 150 atm and a temperature of 50°C unless otherwise noted. The 10 mL reaction cell was made of a 9 cm length of 1/2" O.D. x 0.43" I.D. type 316 stainless steel tubing with 1/4” to 1/16” reducing fittings and 2μm pores size frits (Valco Instruments, Houston, TX). Approximately 0.20-0.25 g of S-P-F or aspen sawdust was packed into the reaction cell, 2 mL of acid was added, the reactor was sealed, and the system was heated and pressurized with carbon dioxide. Several acids were utilized (including: hydrochloric acid, sulfuric acid, and acetic acid) at a variety of acid concentrations (including: 1%, 2%, and 5%). Extraction times varied from 4 min. to 1 hr.

Sonication Reactions

Sonication reactions were performed with a 750-watt ultrasonic processor, VCX-750, purchased from Sonics and Materials (Newton, CT). Approximately 0.20-0.25 g of S-P-F or aspen sawdust was suspended in a liquid medium of 12 mL acid in a glass Suslick vessel. The vessel screws onto the threaded end of full-wave probe with a diameter of ½” (13 mm) and a length of 10” (254 mm). The sonication reactions were conducted at 33% of maximum allowed amplitude. The temperature during the reactions was ~50°C – no external heat was applied to the reaction. Several acids were utilized (including: hydrochloric acid, sulfuric acid, acetic acid, and commercially purchased food grade vinegar) at a variety of acid concentrations (including: 1%, 2%, and 5%). Extraction times varied from 4 min. to 1 hr.

Combination of Supercritical Fluid Extractor and Sonicator

The Suprex Prepmaster was modified slightly to allow for simultaneous supercritical carbon dioxide treatment and sonication of the samples; however, it was determined that this configuration was not able to provide a sufficient transfer of sonication energy to the interior of the stainless steel reactor. Therefore, the majority of the experiments were performed either with supercritical carbon dioxide treatment or with sonication, but not with both treatments simultaneously.
**TOC Determination of Reaction Products**

Initial experiments employed a Shimadzu TOC analyzer (Model TOC-V<sub>CSH</sub>) to measure the total organic carbon removed during the supercritical carbon dioxide or sonication experiments. This instrument provides information on the total amount of carbon present in the extracted samples; however, it does not give specific information with regard to the individual sugars that are present in the extracts. Therefore, the majority of reacted samples were analyzed with HPLC and/or GC-MS as described below.

**HPLC Determination of Reaction Products**

After reacting with either supercritical carbon dioxide or sonication, the majority of samples were analyzed with HPLC. This analytical technique allows for the separation and detection of individual monomeric sugars. After reaction, the samples were rinsed with water to remove any free sugars (it was expected that the S-P-F wood sample would produce mainly glucose, while the Aspen wood sample would produce mainly xylose). The resulting aqueous solutions were then neutralized by adding Dowex 2-X8 anion exchange resin (total exchange capacity, dry basis = 4.1 meq/g) that was pretreated with 6 M sodium hydroxide to load the resin with hydroxide ions. The samples were then analyzed using a Shimadzu HPLC. Specific components included: an SLC-10A VP system controller, two LC-10AT VP pumps, and an SPD-10A VP UV-Visible detector set to monitor 190 and 278 nm. The samples were manually injected into an injection valve with a 20 µL sample loop. The column was a 25 cm x 4.6 mm x 5 µm Supelco SUPELCOSIL LC-NH2 column. A 2 cm x 4.0 mm SUPELCOSIL LC-NH2 guard column was installed before the 25 cm main column. The mobile phase was 75% acetonitrile and 25% water with a total flow rate of 1.5 mL/min.

**GC-MS Determination of Reaction Products**

After reacting with either supercritical carbon dioxide or sonication, a few of the samples were also derivatized (to decrease the polarity of any extracted sugars), followed by analysis with GC-MS in an attempt to qualitatively identify any sugars present in the extract. After rinsing the reacted wood samples with water, the samples were filtered, and the filtrate was freeze dried to remove the water. The remaining solid material was mixed with 6 mL pyridine to dissolve any sugars. An aliquot of 2 mL of the pyridine mixture was then mixed with 1 mL of Supelco
BSA+TMCS+TMSI derivatizing reagent. The derivatizing reagent replaces active hydrogens on a molecule (i.e., the hydroxyl hydrogens on a sugar molecule) with trimethylsilyl, Si(CH₃)₃, or TMS. This process decreases the polarity of sugar molecules so that they can be separated and detected using gas chromatographic techniques. A 1 µL aliquot of the sample was then injected into an HP 5890 Series II gas chromatograph with an HP 5971A Mass Selective Detector and an HP-1 12 m x 0.2 mm x 0.33 µm film thickness column. The column was heated from 50°C to 200°C at 10°C/min with a 10 minute hold at 200°C. The injection port temperature was 250°C using a split injection. For identification purposes, full mass spectra were collected.

After reacting with either supercritical carbon dioxide or sonication, many of the samples were also analyzed with GC-MS to monitor the reactions for the presence of furfural and/or hydroxymethyl furfural, which would result from the degradation of individual sugar monomers. Furfural is a degradation product of xylose (which would mainly be present in the Aspen wood sample), and hydroxymethyl furfural is a degradation product of glucose (which would mainly be present in the S-P-F grade wood sample). These reactions are shown in Figure 1. After rinsing the reacted wood samples with water, 2 mL of ether was added to the aqueous samples, the samples were shaken, and the layers were allowed to separate. It was assumed that the vast majority of furfural and/or hydroxymethyl furfural would preferentially partition into the ether layer, while the vast majority of the monomeric sugars would partition into the aqueous layer. A 2 µL aliquot of the ether layer was then injected into an HP 5890 Series II gas chromatograph with an HP 5971A Mass Selective Detector. The GC column was a Supelco SPB 30 m x 0.25 mm x 0.25 µm film thickness column maintained at 75°C for 8 minutes. The injection port temperature was 180°C, and the split ratio was 25:1. For identification purposes, full mass spectra were collected. In other cases, selected ion monitoring (SIM) was used to increase the sensitivity of the analysis.
Figure 1. Degradation of xylose and glucose to form furfural and 5-hydroxymethyl-2-furfural, respectively.

Results and Discussion

Gravimetric Analyses
Wood samples were weighed prior to running the reactions. Reactions were conducted by adding acid, followed by pressurization with carbon dioxide to supercritical conditions or sonication for a specified period of time. For comparison, samples were also mixed with acid and placed in an oven for the same amount of time at the same temperature. After reaction, the remaining wood samples were filtered, dried in an oven, and reweighed to determine the change in mass. Although this change in mass is not necessarily due to extraction of hemicellulose, it can be used to compare the relative ability of each of the treatments to extract some components of the wood.
In general, the sonication treatment removed the greatest mass of wood, followed by supercritical carbon dioxide, followed by reaction in an oven.
One example of this trend is shown in Tables 1 and 2 and Figure 1 for the reaction of aspen and S-P-F grade wood with 1% (0.18 M) sulfuric acid at 50ºC for 30 minutes. As shown for both types of wood, sonication removed more of the wood than supercritical carbon dioxide. Both of these treatments removed more of the wood than simply heating in an oven. These results were very reproducible.

**Table 1.** Reduction in Mass of Aspen after Reacting with 1% H₂SO₄ at 50ºC for 30 min.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mass Before Reaction (g)</th>
<th>Mass After Reaction (g)</th>
<th>Reduction in Wood Mass (g)</th>
<th>% Reduction in Wood Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven</td>
<td>0.2070</td>
<td>0.2015</td>
<td>0.0055</td>
<td>2.7</td>
</tr>
<tr>
<td>Supercritical</td>
<td>0.1956</td>
<td>0.1833</td>
<td>0.0123</td>
<td>6.3</td>
</tr>
<tr>
<td>Sonication</td>
<td>0.2389</td>
<td>0.2140</td>
<td>0.0249</td>
<td>10.4</td>
</tr>
</tbody>
</table>

**Table 2.** Reduction in Mass of S-P-F Wood after Reacting with 1% H₂SO₄ at 50ºC for 30 min.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mass Before Reaction (g)</th>
<th>Mass After Reaction (g)</th>
<th>Reduction in Wood Mass (g)</th>
<th>% Reduction in Wood Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven</td>
<td>0.2190</td>
<td>0.2109</td>
<td>0.0081</td>
<td>3.7</td>
</tr>
<tr>
<td>Supercritical</td>
<td>0.2169</td>
<td>0.2053</td>
<td>0.0116</td>
<td>5.3</td>
</tr>
<tr>
<td>Sonication</td>
<td>0.2848</td>
<td>0.2545</td>
<td>0.0303</td>
<td>10.6</td>
</tr>
</tbody>
</table>

**Figure 2.** Percent reduction in mass after reacting aspen and S-P-F grade wood with 1% (0.18 M) H₂SO₄ at 50ºC for 30 min.
These reactions were also temperature dependent. An example is shown below for the reaction of S-P-F grade wood with supercritical carbon dioxide at 150 atm and varying temperatures. The reactions were conducted for 10 minutes with acetic acid. As can be seen, an increasing mass of wood was extracted as the temperature was increased. Due to instrumental limitations, experiments above 70°C were not conducted as a part of this study.

![Graph showing percent change in mass with temperature](image)

**Figure 3.** Percent reduction in mass after reacting S-P-F grade wood with acetic acid for 10 minutes at various temperatures.

**HPLC Determination of Reaction Products**

A major objective of this study was to evaluate the ability of supercritical carbon dioxide and sonication to extract hemicellulose from wood, yielding monomeric sugars. Therefore, after reacting with either supercritical carbon dioxide or sonication, the majority of samples were analyzed with HPLC. This analytical technique allows for the separation and detection of individual monomeric sugars. Figures 4 and 5 show HPLC chromatograms for standards of the two specific sugars (xylose from aspen and glucose from S-P-F grade) that were expected to be produced in this study. As shown, the HPLC provides good separation and detection of these sugars.
Figure 4. Chromatogram of a xylose standard (3.720 min) analyzed by HPLC.

Figure 5. Chromatogram of a glucose standard (4.637 min) analyzed by HPLC.
Over the course of the project, over 100 separate reactions were run. As mentioned, several parameters were varied; the type of acid used (acetic acid, hydrochloric acid, and sulfuric acid), the strength of the acid, the reaction time, and the reaction temperature were evaluated. Some reactions were conducted with supercritical carbon dioxide, some were conducted with sonication, and some reactions were simply conducted in an oven for comparison purposes. In some cases, HPLC injections of the aqueous extract showed small peaks that matched the retention time of either xylose or glucose. However, these peaks were either too small to adequately detect and quantify or they were not reproduced in future reactions run under identical conditions. Therefore, it is not possible to claim that hemicellulose was indeed converted into monomeric sugars in these experiments. It should be noted that, due to equipment constraints, the reactions performed in this study were done at temperatures that were considerably lower than what is thought to be required for conversion of hemicelluloses to monomeric sugars without the use of supercritical carbon dioxide or sonication. In addition to temperature limitations, the supercritical carbon dioxide reactor and sonicator both consisted of metal components that were in contact with the samples. Therefore, the experiments were also restricted with respect to the concentration of acid that could be used. The majority of experiments were performed with a 1% solution of acid.

**GC-MS Determination of Reaction Products**

An attempt was also made to analyze the extracted samples for the presence of sugars using GC-MS. For this technique to work for polar analytes like sugars, the sugars had to be subjected to a derivatization process prior to injection into the GC-MS. The derivatization process decreases the polarity of the sugar molecules by replacing active hydrogens on the sugar molecules (i.e., the hydroxyl hydrogens) with trimethylsilyl, Si(CH₃)₃, or TMS. This process allows the sugars to be separated and detected using gas chromatographic techniques. For example, Figure 6 shows the total ion chromatogram of a D-xylose standard that was derivatized and analyzed by GC-MS as described in the Experimental Section. Figure 7 shows the mass spectrum of the peak at 9.683 minutes as well as the library mass spectrum of TMS derivatized D-xylose. Monomeric sugar derivatives were not detected in any of the samples tested in this study.
Figure 6. Total ion chromatogram of a D-xylose standard (9.683 min) that was derivatized and analyzed by GC-MS.

Figure 7. Mass spectrum of a TMS derivatized D-xylose standard (top) and the library mass spectrum of TMS derivatized D-xylose (bottom)
Attempts were also made to monitor the reaction extracts for the presence of furfural and 5-hydroxymethyl-furfural which could have resulted from the degradation of D-xylose and D-glucose, respectively (see Figure 1). Monomeric sugars would not have been detected in the reaction extracts (by HPLC or derivatization followed by GC-MS) if the hemicellulose was completely converted into furfural or 5-hydroxymethyl-furfural. After rinsing the reacted wood samples with water, 2 mL of ether was added to the aqueous samples. After separation, an aliquot of the ether layer was introduced into the GC-MS. For identification purposes, full mass spectra were collected. Mass spectra of furfural and 5-hydroxymethyl-2-furfural standards are shown in Figures 8 and 9.

To increase the sensitivity of the analysis, selected ion monitoring (SIM) was also used in an attempt to detect furfural and 5-hydroxymethyl-2-furfural. With aspen reactions, ions 39, 95, and 96 were selectively monitored, and with S-P-F grade wood, ions 53, 97, 109, and 126 were selectively monitored. Furfural was routinely detected in the aspen extracts. Furfural was identified based on a retention time match with a furfural standard as well as by examining the relative ratio of ions 39, 95, and 96. This strongly suggests that some of the hemicellulose from the aspen was broken down into xylose, and the xylose was subsequently converted into furfural. However, the amount of furfural detected only accounted for a very small percentage (<0.1%) of the original mass of wood. In extracts derived from the reaction of S-P-F grade wood, 5-hydroxymethyl-2-furfural was not detected using SIM.

![Mass spectrum of furfural](image.png)

**Figure 8.** Mass spectrum of furfural.
Reactions with Xylose and Arabinogalactan

Two additional experiments were conducted in an attempt to better understand what was happening with the reactions. Rather than starting with aspen or S-P-F grade wood, the effects of performing reactions directly on a pure sugar (xylose) and a longer chained polysaccharide (arabinogalactan) were examined – arabinogalactans are long, densely branched polysaccharides of varying molecular weights (10,000-120,000). In the first set of experiments, ~0.02 g of pure xylose was mixed with various concentrations of sulfuric acid and reacted for 30 minutes at various temperatures. Some reactions were performed with heat alone (in an oven) at a temperature of 85°C using 2% and 5% sulfuric acid. Other reactions were performed with sonication at ~50°C using 1%, 2%, and 5% sulfuric acid. After reaction, the solutions were neutralized with Dowex and analyzed with HPLC. Quantitative analysis showed that ~100% of the xylose remained unreacted during these reactions. In the second set of experiments, pure arabinogalactan was reacted. Upon degradation, arabinogalactan will yield arabinose and galactose monomeric sugars. Figures 10 and 11 show HPLC chromatograms for standards prepared from pure arabinose and galactose. With these experiments, ~0.02 g of arabinogalactan was mixed with 1% and 2% sulfuric acid and reacted for 30 minutes at ~50°C using sonication. After reaction, the solutions were neutralized with Dowex and analyzed with HPLC. Neither arabinose nor galactose was detected as products of these reactions.

Figure 9. Mass spectrum of 5-hydroxymethyl-2-furfural.
Figure 10. Chromatogram of an arabinose standard analyzed by HPLC.

Figure 7. Chromatogram of a galactose standard analyzed by HPLC.
Conclusions

Although this study did not result in the detection of pure sugars released from either aspen or S-P-F grade wood, there were a number of useful outcomes. First, it was consistently demonstrated that reactions conducted with acid combined with sonication or supercritical carbon dioxide removed a larger mass of wood than by treating with acid alone, even at a relatively low temperature (~50°C). It should be noted that this change in mass is not necessarily due to extraction of hemicellulose. In general, the sonication treatment removed the greatest mass of wood, followed by supercritical carbon dioxide, followed by reaction in an oven. There was also a significant increase in the mass removed with increasing reaction temperature. In addition, some furfural was positively identified as a reaction byproduct from reactions with aspen; however, the mass of furfural was very small when compared with the initial mass of wood (<0.1%). This does provide evidence that some hemicellulose was extracted, converted to xylose, and finally converted to furfural. It is possible that even more furfural was produced but was lost to volatilization either during the reaction or during subsequent processing. Due to experimental limitations, reactions with sonication or supercritical carbon dioxide were not conducted above 70°C; however, it is expected that if the temperature were to be increased higher, significant amounts of products (i.e., sugars and/or aldehyde byproducts) would be produced. Future research will be focused on constructing an experimental set up that will allow for higher temperature reactions to be conducted.
References Cited