REMOVAL OF ACID-SOLUBLE LIGNIN FROM BIOMASS EXTRACTS USING AMBERLITE XAD-4 RESIN

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This paper describes a method for the removal of acid-soluble lignin from acid-hydrolyzed hemicelluloses extracted from a mixture of northern US hardwood chips, by using Amberlite XAD-4 resin, which was shown to remove 100% of furan derivatives and 90% of acid-soluble lignin. Subsequent fermentation of the resin-treated hydrolyzates gave ethanol yields as high as 97% of theoretical and showed a marked increase in the fermentation rate. Regeneration of resin performed with 75% acetone was 85% efficient with respect to acid-soluble lignin.

Keywords: Acid-soluble lignin; XAD-4; Biomass pretreatment; Green liquor extraction; Hot water extraction; Fermentation inhibitors

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INTRODUCTION

The era of readily available, low cost petroleum products seems to be behind us, with forecasts indicating that we have either neared or surpassed peak oil production (Nashawi et al. 2010). Furthermore, the combustion of fossil fuels is responsible for the release into the atmosphere of seven billion tons per year of carbon dioxide (Zhang 2008). There is, then, a pressing need to reduce our dependence on petroleum by making use of alternative fuel and chemical feedstocks. One of the most promising of these is lignocellulosic biomass, and it is the only feedstock readily available to convert to a liquid fuel (Huber et al. 2006). According to the US Department of Energy, enough biomass exists in the United States, without significant modification to present infrastructure, to supplant more than one third of US petroleum consumption (Perlack et al. 2005).

The forest products industry has many years of experience with the unique problems of biomass handling. While pulping residue is presently burned for energy, hemicellulose does not have a high energy content relative to that of lignin, comprising only about 25% of the energy in the pulping residue (Ragauskas et al. 2006). As such, it has been proposed to extract and hydrolyze hemicellulose prior to pulping and convert the resulting sugars to alcohols or other biofuels (van Heiningen 2006; Mao et al. 2008).

The alkaline extraction process is not perfect, and degradation of monosugars can lead to furfural and hydroxymethylfurfural (HMF) in the extracts (Garrote et al. 1999). Some residual lignin is co-extracted as well. While a fraction of the lignin, known as Klason lignin, is precipitated during conventional acid hydrolysis and can be removed by filtration, acid-soluble lignin remains in solution largely as phenolic compounds of low

molar mass (Yasuda et al. 2001). These phenolics are detrimental to fermentation by microorganisms (Cantarella et al. 2000; Fenske et al. 1998; Klinke et al. 2004; Nilvebrandt et al. 2001; Palmqvist and Hahn-Hagerdal 2000; Pienkos and Zhan 2009). The effects are magnified by the presence of furfural and its derivatives, which are present in the hydrolyzates as degradation products of xylose and glucose (Zaldivar et al. 1999). Various methods have been described for the removal of furan derivatives from biomass hydrolyzates, including the use of enzymes, evaporation, extraction on diethyl ether, overliming, and adsorption on ion-exchange resins (Cantarella et al. 2000; Nilvebrandt et al. 2001; Palmqvist and Hahn-Hagerdal 2000; Pienkos and Zhan 2009); however these results have not always been extended to removal of lignin monomers or oligomers.

Amberlite XAD-4 is a styrene-divinyl benzene hydrophobic interaction resin manufactured by Dow Chemical (formerly Rohm & Haas) and has been shown to be effective in removing furfural from corn stover hydrolyzates and SPORL-treated lodgepole pine (Weil et al. 2002; Zhu et al. 2010). The resin has also been used somewhat successfully for removal of organic acids from corn fiber hydrolyzates (Ezeji et al. 2007) as well as for low levels of phenolics when used in conjunction with activated carbon (Kim et al. 2005). This paper explores the use of XAD-4 for the adsorption of such compounds – specifically acid-soluble lignin - and includes the effect of solution pH. A method for the regeneration of the resin is reported, as are the effects of acid-soluble lignin removal on extract fermentability.

EXPERIMENTAL

Materials

XAD-4 resin was purchased from Acros Organics (CAS 9003-69-4). Adsorption columns were made using 10 mL plastic chromatography columns purchased from Bio-Rad. This resin was washed three times with methanol (one resin bed volume) and then rinsed with 10 resin bed volumes of deionized water to remove any impurities, as per Rohm and Haas' recommendations. Hemicellulose was extracted from mixed northern US hardwood chips as described below. All other chemicals were used as received and were of analytical grade.

Resin Characterization

The adsorption of acid-soluble lignin from solution was performed using 10 mL plastic chromatography columns to contain a resin bed of approximately 4 g of XAD-4 resin. Adsorption was performed in the small columns by retaining 5 mL of solution in the resin bed for 10 minutes prior to elution.

Hemicellulose Extraction

The ability of the resin to remove acid-soluble lignin from solution was made using hot water extracts of mixed northern hardwood chips. Alkaline extraction was performed using 2% green liquor (a mixture of sodium sulfide and sodium carbonate that is an intermediate in the kraft pulping recovery cycle) at an H-factor (Vroom 1957) of

600 and a liquor to wood ratio of 4:1. Hot water extraction was also performed at an H-factor of 600 with the same liquor to wood ratio. The extracts were then hydrolyzed using 3% sulfuric acid at 121 ºC for 1 hour. Klason lignin was filtered, washed with hot water, dried, and weighed. The procedure, using slightly different extraction conditions, is detailed elsewhere. (Um and van Walsum 2009, 2010; Walton et al. 2010). Detoxification using the XAD-4 resin was performed by retaining 5 mL of extracts on a 4 g column for 10 minutes. The extracts were drained from the column and retained again for an additional 5 minutes.

Column Regeneration

The saturated resin was regenerated using acetone. In order to determine the necessary concentration of acetone, three volumetric concentrations were tested: 20 vol%, 50 vol%, and 75 vol%. 15 mL of acetone solution were used for every gram of resin. The acetone was collected in three equal fractions and was analyzed for furan derivatives and acid-soluble lignin as detailed below.

Fermentation

Improvements in the fermentability of the detoxified extracts were studied using *E. coli* K011. Hot water extracts were treated in a 150 g resin bed contained in a 500 mL separatory funnel with a glass wool plug above the stopcock. The procedure was similar to that described above; however, the second retention of the extracts was increased from 5 to 10 minutes. Further, the resin column was rinsed with two resin bed volumes of water to recover any remaining sugars. The wash water was mixed with the detoxified extracts and rotary evaporated to remove the excess wash water. The extracts were then neutralized using calcium hydroxide and the resulting gypsum filtered and removed.

Fermentation was performed in 125 mL serum vials. The extracts were sterilized and prepared for bacterial growth by the addition of 25 g/L LB medium, 40 mg/L chloramphenicol (an antibiotic), 5 mL/L of a trace metals solution, and 1 mg/L of thiamine. The trace metals solution contained 5 g/L disodium EDTA, 0.22 mg/L zinc sulfate heptahydrate, 0.5 g/L calcium chloride, 0.4 g/L ferrous sulfate, 0.1 g/L ammonium molybdate tetrahydrate, 0.16 g/L cupric chloride, and 0.16 g/L cobalt chloride. 50 mL of the extracts were inoculated with *E. coli* K011 at 5% of the working volume and were incubated at 37 ºC and 200 RPM in a New Brunswick Scientific shaker. Samples were taken over a period of several hours to determine ethanol and sugar concentrations.

Chemical Analysis

Furfural concentration was analyzed using quantitative gas chromatography/mass spectrometry (GC/MS). A Shimadzu (Columbia, MD) GC2010 gas chromatograph equipped with a GCMS-QP2010S quadrupole mass spectrometer and a Shimadzu SHRX1-5MS column was used. The aqueous solutions were extracted with HPLC grade dichloromethane and dried over anhydrous sodium sulfate. Phenol was used as an internal standard. A detector response curve was generated using four different concentrations of reagent grade furfural dissolved in deionized water. The GC program was as follows: oven initially at 40 ºC, ramped to 120 ºC at 3 ºC/min and held for 2 minutes, ramped to 180 ºC at 5 ºC/min and held for 5 minutes, ramped to 220 ºC at 25

ºC/min and held for 10 min. Compounds were identified by comparison with NIST library mass spectra provided with Shimadzu's GCMSsolution software.

Klason lignin content was measured gravimetrically. Acid-soluble lignin content was determined using TAPPI Useful Method UM 250. (1985) The samples were diluted to give a measured absorbance at 205 nm of between 0.2 and 0.7. A Thermo-Electron Corp. Evolution 100 UV-Vis spectrophotometer with 1cm path length quartz cells was used. Equation 1 calculates the concentration of acid-soluble lignin in g/L.

$$ASL = \frac{A_{205} \cdot f}{a \cdot l} \quad (1)$$

In Equation 1, *ASL* corresponds to the acid-soluble lignin concentration in g/L, A_{205} corresponds to the absorbance at 205 nm, *f* corresponds to the dilution factor, and *l* corresponds to the path length. An absorptivity, *a*, of 113 L/g-cm was used to correspond to hardwood extracts.

Analysis of fermentation samples was performed on a Shimadzu (Columbia, MD) high performance liquid chromatograph equipped with refractive index and UV detectors as well as an Aminex HPX-87H column (Bio-Rad, Hercules, CA). The column was operated with a 5 mm sulfuric acid mobile phase at 0.6 mL/min and 60 ºC. Samples were filtered through 0.22 μm syringe filters prior to injection.

RESULTS AND DISCUSSION

Resin Characterization

Because the behavior of XAD-4 resin has already been well documented when removing furfural from aqueous solution (Nanoti et al. 2008; Weil et al. 2002), optimization of the working pH was performed using a furfural solution of 1 g/L. Furfural was diluted using deionized water, and the pH was reduced to mimic various process conditions at pH 1, pH 5, and pH 7 using sulfuric acid. The best operating pH was found at a value of 1 based on Fig. 1. At pH 1, 99% of the furfural was adsorbed on the column, while only 85% and 70% was adsorbed at pH 5 and 7, respectively. This acidic condition is consistent with previous reports (Weil et al. 2002; Zhu et al. 2010). From a process design standpoint, it is convenient that pH 1 be the optimal working pH, as acid hydrolysis is carried out at these rigorous conditions, and consequently, an XAD-4 adsorption column would need no preconditioning steps.

Hemicellulose solutions were prepared using well-documented green liquor and hot water extraction processes (Um and van Walsum 2009, 2010; Walton et al. 2010). Both the hot water and green liquor extractions were carried out at H-factors of 600 and liquor to wood ratios of 4:1, as described above. The lignin content of the extracts was measured gravimetrically for Klason lignin and spectrophotometrically for acid-soluble lignin (TAPPI 1985). Table 1 shows the lignin content as measured for the two extraction conditions. Total soluble lignin is the sum of the acid-soluble lignin and klason lignin. The higher pH of the green liquor extracts stems from the alkaline buffering capacity of green liquor towards organic acids produced during the extraction.

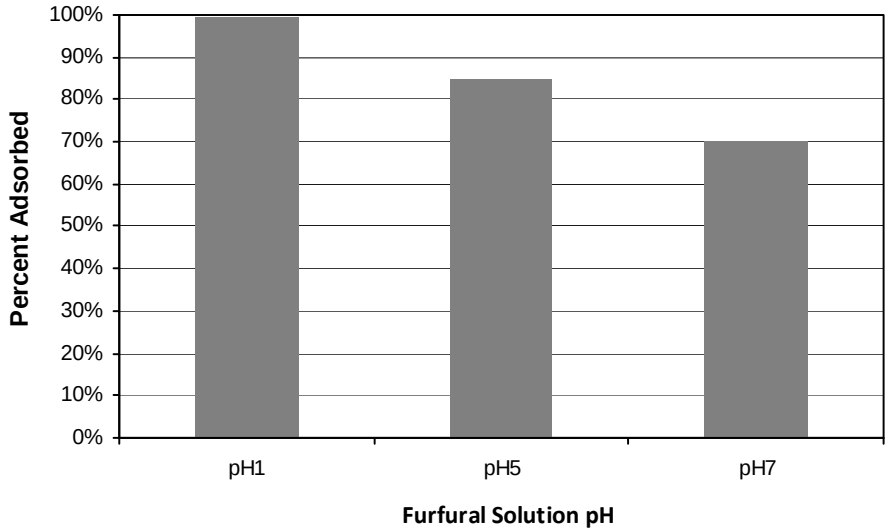

Fig. 1. Percent of furfural adsorbed at varying pH

Table 1. Lignin Content of Extracts

	H-Factor	*Acid-Soluble Lignin (g/L)*	*Klason Lignin (g/L)*	*Total Soluble Lignin (g/L)*	*pH*
G.L. Extracts	600	2.24	2.03	4.27	5.45
Hot Water Extracts	600	2.55	3.27	5.82	3.73

GCMS analysis of the acid hydrolyzed hot water extracts and acid hydrolyzed green liquor extracts showed a variety of furan derivatives and monomeric phenolic compounds. These monomers are identified in Table 2.

While the literature indicates that hexose concentrations from SPORL-pretreated lodgepole pine are not significantly affected by XAD-4 treatment (Zhu et al. 2010), it was observed that the resin did retain a portion of the extracted monosugars after batch adsorption, as shown in Table 3. This is likely the result of some of the extracts becoming trapped in the bulk void space of the resin bed. To support this hypothesis, a simple water wash recovered more than 90% of the glucose, xylose, mannose, and galactose. Only 81% of the arabinose was recovered by the water wash. The reason for the lower recovery of arabinose is still not known, and further work is required to establish the exact mechanisms involved.

The hemicellulose extracts were passed through adsorption columns containing XAD-4 resin as a method of detoxification. This treatment with XAD-4 resin completely removed all of the compounds identified in Table 2 from both the green liquor and hot water extracts. Also, a 90-92% decrease in acid-soluble lignin concentration in both extracts was observed (calculated from data in Table 3). Acetic acid produced from the de-acetylation of 4-O-methylglucuronoxylan during extraction (Brasch and Free 1964), as well as formic acid, a sugar degradation product, were also analyzed (Table 3). Almost complete recovery of formic acid from the resin was achieved after 2 consecutive water washes, while about 20% of the acetic acid was retained on the resin.

Table 2. Identified Furan Derivatives and Phenolic Monomers in Each Extract

	Acid Hydrolyzed GL Extracts	*Acid Hydrolyzed Hot Water Extracts*
Furfural	*x*	*x*
5-methyl-2 furancarboxaldehyde	*x*	*x*
Succinic anhydride	*x*	*x*
Fufuryl alcohol	*x*	
2,5-Furandicarboxaldehyde		*x*
Hydroxymethylfurfural	*x*	*x*
Guaiacol	*x*	*x*
2,6-Dimethoxyphenol	*x*	*x*
Vanillin	*x*	*x*
Acetoguaiacone	*x*	*x*
Methyl-(2-hydroxy-3ethoxy-benzyl)ether	*x*	
Syringaldehyde	*x*	
Vanillacetic acid	*x*	
4-Hydroxy-2methoxycinnamaldehyde	*x*	
Guaiacylacetone	*x*	
2,6-Dimethoxy-4-[-1-propenyl]phenylacetate	*x*	
Vinylguaiacol	*x*	
Acetoeugenol	*x*	
3,4,5-Trimethoxyphenol	*x*	
X = compound was identified		

Table 3. HPLC Analysis of Acid-Hydrolyzed Hot Water Extracts Before and After Adsorption on XAD-4. Also shown are two water washes used to recover monosugars. Masses are per 250 mL of acid hydrolyzed extracts.

	Glucose (g)	*Xylose (XMG) (g)*	*Arabinose (g)*	*Formic Acid (g)*	*Acetic Acid (g)*	*Acid-Soluble Lignin (g)*
Pre-XAD-4	0.69	4.88	0.27	0.10	1.27	0.54
Post-XAD-4	0.51	2.56	0.12	0.06	0.51	0.05
1st Water Wash	0.10	1.53	0.09	0.02	0.16	0.01
2nd Water Wash	0.04	0.60	0.01	0.02	0.36	0.01
% Recovered	93.0%	96.0%	81.9%	95.7%	80.9%	12.2%

From a technical standpoint, it is crucial that the resin can be regenerated for re-use for in economical process. Thus, we attempted to regenerate the resin with several solvents. The resin could not be regenerated with ethanol, as has been reported in its use with furan derivatives (Weil et al. 2002). Acetone was shown to much more completely regenerate the resin, as expressed in Fig. 2. The amount desorbed showed an optimum at an acetone concentration of 75 vol%.

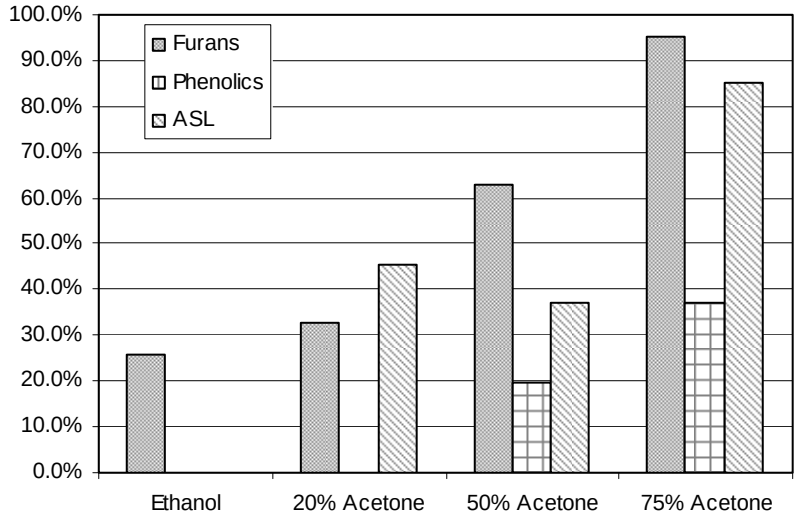

Fig. 2. Recovery of furans, phenolic monomers, and acid-soluble lignin (ASL) using various solvents. Note that the ability of ethanol to remove ASL was not investigated.

Removal of acid-soluble lignin from acid hydrolyzed hot water extracts was shown to have a significant impact on the fermentability of the extracts. The concentration of ethanol that can be produced under ideal conditions is 51% of the concentration of sugar that is consumed, based on the stoichiometry of the fermentation reaction. While stock extracts without detoxification can show fermentation yields as high as 80% of this theoretical yield, this is highly dependent on microbial growth. During the course of this study, for example, only one of five innoculations of untreated acid hydrolyzed hot water extracts showed any growth. The untreated extracts contained 2.16 g/L of acid-soluble lignin, while the XAD-4 treated extracts only contained 0.28 g/L of acid-soluble lignin.

After the removal of inhibitors, yields between 87.5% and 97% of theoretical yield were observed. In the studies performed, it was also observed that fermentation was much more consistent in proceeding to completion. Yields of approximately 90% of theoretical yield are consistent with the literature reports (Weil et al. 2002; Zhu et al. 2010). In their study, Weil et al (2002) reported that fermentation of XAD-4 treated corn fiber hydrolysates approached 90% of theoretical yield.

The rate of fermentation was also qualitatively observed to improve after adsorption on XAD-4 resin, as seen in the variations of sugar and ethanol concentration in Fig. 3. After approximately 30 hours, ethanol production and xylose / mannose / galactose (XMG) consumption in XAD-4 treated samples had approached a stable value, indicating almost complete conversion of sugar to ethanol. Conversely, in untreated samples, ethanol production and XMG consumption did not begin until 24 hours after inoculation. 72 hours after inoculation, full conversion still had not been reached. In the untreated case, the initial sugar concentration was approximately 14 g/L, while in the treated case the initial sugar concentration was 16 g/L. However, similar increases in rate and yield were noted when fermenting an XAD-4 treated sample with an initial sugar concentration of 9.5 g/L.

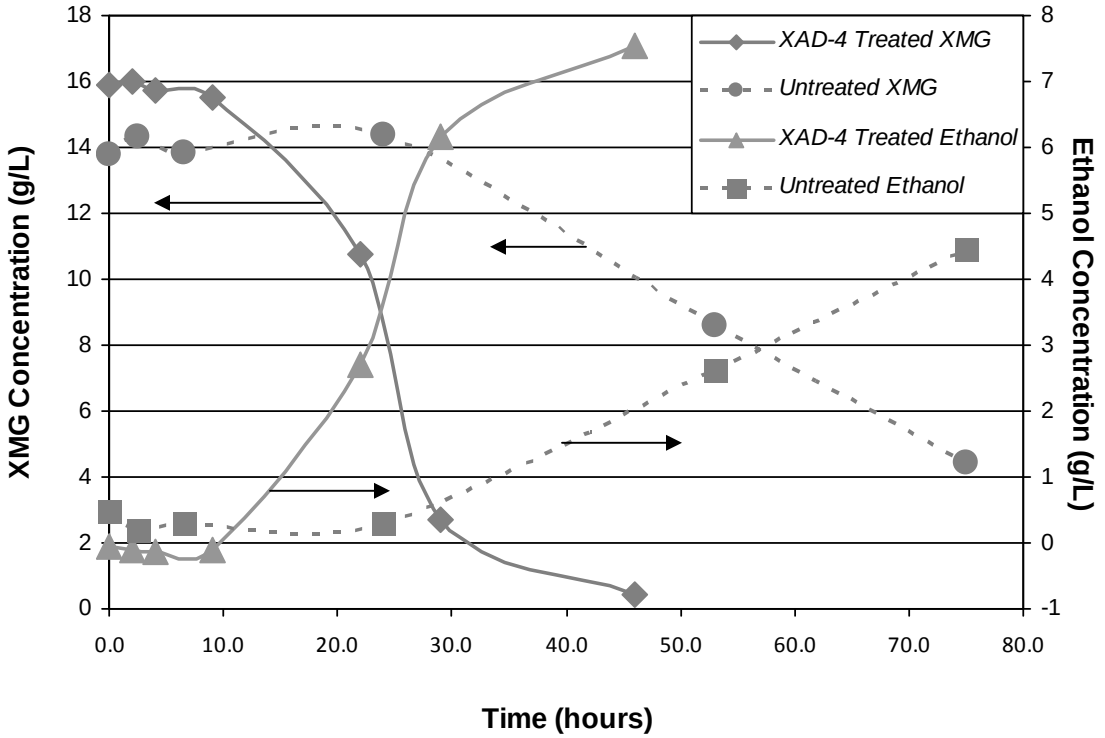

Fig. 3. Consumption of xylose/mannose/galactose and ethanol production in both XAD-4 treated (solid lines) and untreated (dashed lines) samples. Arrows indicate which plot accompanies which axis.

It should be noted that, in addition to increases in the rate of fermentation, the removal of acid-soluble lignin also improved the success rate of fermentation. Five untreated samples of acid hydrolyzed hot water extracts were inoculated; however of those five only one showed microbial growth. Conversely, six XAD-4 treated samples were fermented, and all six produced ethanol at the increased rate noted above.

Increases in the rate of fermentation are consistent with reports in the literature. Zaldivar et al. (1999) report that the growth of *E. coli* K011 decreases with increased concentrations of both furan derivatives and phenolic aldehydes. Weil et al (2002) also report increases in the rate of fermentation upon removal of furfural from hydrolyzed corn fiber. The removal of these inhibitors would be expected to improve the ability of the cells to grow and, hence, produce ethanol. Such an increase is significant when considering the economics of a commercial fermentation process. A faster reaction would require a smaller reactor, thereby decreasing the capital cost associated with this branch of biorefining.

CONCLUSIONS

1. Increases in the fermentability of biomass extracts are possible by removal of acid-soluble lignin. Specifically, an improved yield and faster rates make any

fermentation process more economical by virtue of requiring smaller, cheaper processing equipment.
2. Amberlite XAD-4 resin is a suitable adsorbent for the detoxification of biomass extracts that contain acid-soluble lignin. The ideal pH for adsorption is pH 1, which eliminates any need for conditioning prior to detoxification.
3. The resin can be regenerated at ambient temperatures using acetone solution. The optimal acetone concentration for removal of acid-soluble lignin and furan derivatives is 75 vol%.
4. The removal of furfural and acid-soluble lignin results in a significant increase in both the yield and rate of fermentation of biomass extracts.

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