Oligomer saccharide reduction during dilute acid pretreatment co-catalyzed with Lewis acids on corn stover biomass

John Degenstein[1], Srinivas Reddy Kamireddy[2], Melvin P. Tucker[3], Yun Ji[2*]

(1. *Department of Chemical Engineering, Purdue University, West Lafayette, Indiana 47907, USA*;
2. *Department of Chemical Engineering, University of North Dakota, Grand Forks, North Dakota 58202, USA*;
3. *National Renewable Energy Laboratory, Golden, Colorado 80401, USA*)

Abstract: The dilute sulfuric acid pretreatment of lignocellulosic biomass is a well understood process that significantly enhances the yield of glucose after enzymatic saccharification. The goal of this research was to perform a systematic study to evaluate the yield of fermentable sugars during dilute sulfuric acid pretreatment that is co-catalyzed with the transition metal Lewis acid salts: $AlCl_3$, $FeCl_2$, $FeCl_3$, and $La(OTf)_3$. All Lewis acids apart from $FeCl_2$ reduced the presence of xylo-oligomers by a large margin when compared to the non-co-catalyzed control sample pretreatments. The presence of these xylo-oligomers acts as inhibitors during enzymatic saccaharification step. The Lewis acids $AlCl_3$, $FeCl_3$, and $La(OTf)_3$ were also able to marginally increase the overall enzymatic digestibility specifically for corn stover pretreated at 160 ℃ with 10 mM of Lewis acids. The hard Lewis acid such as $AlCl_3$ increased the formation inhibitory products such as furfural and 5-hydroxymethylfurfural (HMF). There was good correlation between reduction of xylo-oligomers and increased concentration furfural with increase in Lewis acid hardness.

Keywords: pretreatment, corn stover, biomass, biofuel, enzymatic saccharification, Lewis acid, transition metal
DOI: 10.3965/j.ijabe.20130602.007

Citation: Degenstein J, Kamireddy S R, Tucker M P, Ji Y. Oligomer saccharide reduction during dilute acid pretreatment co-catalyzed with Lewis acids on corn stover biomass. Int J Agric & Biol Eng, 2013; 6(2): 54－62.

1 Introduction

The production of fuels and green chemicals from widely available renewable lignocellulosic biomass is an important step towards domestic energy independence as well as reduction in carbon output[1]. One way of accomplishing this goal is by pretreatment of biomass followed by enzymatic saccharification and fermentation[2]. Lignocellulosic biomass primarily consists of cellulose, hemicellulose, and lignin. Cellulose consists of organized microfibrils, each consisting of 3-6 nm in diameter that has thousands of six carbon monomers of glucose[3]. Hemicellulose is a hetero polymer consists of five and six carbon carbohydrate molecules in the form of xylose, galactose, arabinose, mannose, and glucose[3]. Lignin is a complex hydrophobic polymer of p-hydroxyphenyl, guaiacyl, and syringyl residues that fills in the spaces between the cellulose fibers and hemicellulose[4].

Primarily pretreatment is performed to overcome the recalcitrant nature of the biomass due to presence of hemicellulose and lignin. There are several paths to perform pretreatment such as physical, liquid hot water, steam explosion, and chemical pretreatment (acid or alkali)[3]. Hence, pretreatment is considered as one of

Received date: 2013-02-22 **Accepted date:** 2013-04-26
Biographies: John Degenstein, PhD Student, Department of Chemical Engineering, Purdue University; Email: jdegenst@purdue.edu. **Srinivas Reddy Kamireddy,** PhD student, Department of Chemical Engineering, University of North Dakota; Email: kamireddy.srinu@gmail.com. **Melvin P. Tucker,** Senior Research Engineer, National Bioenergy Center, National Renewable Energy Laboratory, 1617 Cole Blvd; Email: melvin.tucker@nrel.gov.
***Corresponding author: Yun Ji**, Assistant Professor, Department of Chemical Engineering, University of North Dakota, 241 Centennial Drive, Grand Forks, ND 58202, USA. Tel: 701-777-4456; Email: yun.ji@engr.und.edu.

the most expensive unit operation steps in the conversion of raw biomass into fermentable sugars[4-5]. The dilute sulfuric acid pretreatment was employed in this study as it was found to be very economical and efficient[6]. The acid solution primarily acts on branched structure of amorphous hemicellulose and cleaves the acetyl linkages and converts hemicellulose into individual monomers[7]. This results in exposing crystalline structure of cellulose as it enhances the porosity and surface area. This in turn increases the fermentable sugar yields during enzymatic saccharfication[7].

An optimum condition of pretreating corn stover biomass was found to be between 170℃ and 180℃ at > 1% (w.t.) sulfuric acid concentration for 8-10 min[8]. Conversely, at this condition, the degradation of xylose into furfural was found to be greater than 15% (w.t.). The higher concentration of furfural adversely influences the enzymatic saccharification and fermentation yields[9]. This issue can be resolved by performing pretreatments at lower acid concentration coupled with lower reaction temperatures (low severity). However, at low severity pretreatments can lead higher amount of xylooliogmers in the pretreated liquid hydrolyzate samples. These oligomers can also act as strong inhibitors during enzymatic saccharification by cellulase enzymes as evident from study conducted by Qing et al[10]. In general, in order to de-polymerize these oligomers an additional acid hydrolysis step has to be employed after the pretreatment on liquid hydrolyzate slurry solutions[11]. This may lead to further degradation of fermentable sugars and also incur additional cost on the operation of the bio-process plant.

The recent study conducted by Wei et al.[12] revealed the addition of 5 mM concentration Fe^{2+} ion in the form of $FeSO_4$ salt acted as co-catalysts in dilute acid pretreatment, as there was an increase in the yield of glucose during enzymatic saccharification[12]. As an extension to this work we decided to employ several new co-catalysts, mainly Lewis acid salts, with varying concentration in the dilute-acid pretreatment of corn stover. The four Lewis acids used in the study were $FeCl_2$, $FeCl_3$, $AlCl_3$, and $La(OTf)_3$.

The goal of this research is to validate whether the addition of these Lewis acids in mM concentrations has any significant improvement in the reduction of oligomers, and its subsequent effects on the enzymatic digestibility and formation of inhibitor products. In this way we can eliminate the secondary hydrolysis step that may reduce the cost of operation of the bio-process plant. Another goal of this study is to find whether there is any correlation between different Lewis acid chemical hardness and its effects on oligomers and degradation products, which is mainly furfural.

2 Materials and methods

2.1 Feedstock materials

The feed stock materials were provided by the National Renewable Energy Laboratory (NREL) (Golden, CO). Corn stover was harvested from Wray, CO and milled to 1/4 inch size. The composition of corn stover was analyzed at NREL. It contains 33.4% cellulose, 21.8% hemicellulose, 11.2% lignin, 3.7% ash, and 9.3% extractives by dry weight[13].

2.2 Reactor setup

A batch reactor was used to perform the pretreatments. This reactor system is based upon the 300 mL EZE-Seal jacketed reactor made by Autoclave Engineers (Erie, PA). In order to mitigate the effect of dissolved ions during pretreatments as well as to reduce corrosion, the wetted parts of this reactor were made from Hastelloy RC-276. A 3 kW Sussman steam generator with a custom built steam accumulation drum provides fast heating kinetics for the lignocellulosic biomass to reach a desired temperature. The steam accumulation drum is necessary for the system to provide efficient operable dynamics given in a relatively small internal volume of the steam generator itself. The volume of the steam accumulation drum is 30 L. The steam accumulation drum is well insulated and equipped with a bottom reboiler to maintain the steam temperature. The steam generator is rated for a maximum operating pressure of 689.4 kPa which corresponds to a maximum steam temperature at 166℃. The average heating kinetics of the reactor was around 35℃/min. The agitation was performed by magnetic motor and was maintained constant at 60 r/min throughout the reaction period. Steam was injected into

the external jacket of the reactor from the boiler by operating a three-way valve manually. Once the desired temperature was reached, reaction time was initiated. After the desired reaction time, steam was shut off and cooling water was pumped into the external jacket of the reactor. Once the reactor was cooled down below 40 ℃, slurry samples were withdrawn from the reactor into polyethylene bottles and stored in refrigerator for further analysis. Additional information concerning the reactor system is available in a previously published paper[14].

2.3 Analytical procedures

Pretreated slurry samples were filtered under vacuum and separated into solids and liquid fractions. The liquid fraction was analyzed for monosaccharides and fermentation inhibitor products. This analysis was performed based on the NREL analytical procedures (NREL/TP-510-42623). The solid fraction was analyzed for cellulose, hemicellulose, acid insoluble lignin, acid soluble lignin, and ash contents based on NREL/TP 510-42618, "Determination of Structural Carbohydrates and Lignin in Biomass". A quantitative analysis for determining monosaccharides present in liquid fraction was performed by Agilent 1200 HPLC (Palo Alto, CA) with Transgenomic CHO-Pb carbohydrate separation column length 300 × 7.8 mm (Omaha, NE). All samples were replicated and analyzed by HPLC. The mobile phase used for analysis was deionized water with a flow rate of 0.6 mL/min. Prior to analyzing pretreated hydrolyzate samples, a set of calibration standards were run to validate the HPLC RID. The concentrations of the standards were ranged from 0.5 g/L to 18 g/L. In addition, internal sugar recovery standards with a concentration of 4 g/L was run frequently (every 8 injections) to test for column and RID validity. The standard solutions of sugar recovery standard solution consist of D-(+) glucose, D-(+) xylose, D-(+) galactose, L-(+) arabinose, and D-(+) mannose. In addition, due to the presence of a large amount of carbohydrate oligomers in many samples an additional 4% (w.t.) secondary acid hydrolysis at 121 ℃ was performed on the slurry liquor to quantify the amount of total sugar present in the samples[15].

Inhibitor products were analyzed using an Agilent 1200 HPLC with Phenomenex Rezex RFQ 100 × 7.8 mm column (Torrance, CA). The 0.01 N sulfuric acid mobile phase with a flow rate of 1 mL/min was used for analysis[15]. The verification standards for fermentation inhibitor products were obtained from Absolute Standards, Inc (Hamden, CT).

2.4 Enzymatic saccharification

The pretreatment enzymatic hydrolysis was performed in duplicate for each pretreatment experiment (for a total of 104 enzymatic hydrolysis runs). Substrate blanks were also performed on the control experiments only (runs without enzymes) and enzyme blanks (runs without solids). The enzymatic hydrolysis of the cellulose substrate was performed in a thermal incubator (Thermo Scientific, MaxQ 4000) at 50 ℃ and 220 r/min for 72 h. Hydrolysis was performed with sodium citrate buffer (Sigma Aldrich, St.Louis, MO) with 50 mM/L concentration (pH of 4.8) and sodium azide (Sigma Aldrich, St.Louis, MO) with a concentration of 20 mg/mL. These reagents along with deionized water were added so that substrate accounts for 2% (w.t.) of cellulose. A cellulase enzyme, commercially known as GC 220 (Genencor, Palo Alto CA), was used to perform the enzymatic hydrolysis. Fifty four milligrams of cellulose enzyme of per gram of protein of loading was used to perform the enzymatic hydrolysis. These optimized enzyme loading conditions were based on our previous studies on sunflower hulls and sugarbeet[16,17]. After hydrolysis, the liquid hydrolyzate samples were filtered using 0.2 μm porous nylon syringe filters from Millipore (Billercia, MA) into glass vials manufactured from Agilent (Palo Alto, CA). In order to deactivate the enzymes after saccharification, all the vials were stored in freezer at -20 ℃ for 24 h. The vials were then removed from the freezer and brought to room temperature to analyze for glucose concentration by Agilent 1200 HPLC (Palo Alto, CA) system with Transgenomic CHO-782 Pb (Omaha, NE) carbohydrate separating column. Enzymatic digestibility was calculated using Equation (1). The hydration correction factor of 0.9 was used to analyze the enzymatic digestibility. This procedure is based on the NREL LAP protocol (NREL/TP 510-42629).

$$\%\ \text{Digestion} = \frac{\text{Grams of cellulose digested} \times 0.9 \times 100}{\text{Grams of cellulose added}} \quad (1)$$

2.5 Lewis acids selection

For this study the following four Lewis acids were studied: $FeCl_2$, $FeCl_3$, $AlCl_3$, and $La(OTf)_3$. Iron (II) Chloride was chosen as a baseline comparison to the existing patent[18]. Iron (III) Chloride was chosen as a comparison to Iron (II) Chloride. Aluminum Chloride was chosen because of its reputation as a very strong Lewis acid[19]. Lanthanum Trifluoromethane-sulfonate (Lanthanum Triflate) was finally chosen due to its selectivity combined with its strong activity in several aqueous organic reactions[20]. In addition, the concentration of Lewis acids ranged from 1 mM to 10 mM as evident from Table 1. The primary reason for choosing such low concentration is to avoid degradation of fermentable sugars during pretreatment and also to make the process economical as these salts are expensive.

Table 1 Experimental design for the Lewis acid co-catalyzed dilute sulfuric acid pretreatment of corn stover. The runs including a Lewis acid were repeated for each of $FeCl_2$, $FeCl_3$, $AlCl_3$, and $La(OTf)_3$

Repeats	Temperature/℃	Lewis acid concentration/mM
2	140	1
2	140	10
2	150	5.5
2	160	1
2	160	10
4	140	0 (Control run)
4	150	0 (Control run)
4	160	0 (Control run)

The reaction conditions were chosen to represent a reasonable level of pretreatment severity, which are potential candidates in the impending commercialization of the dilute-acid pretreatment. For example, the current NREL process design for biochemical conversion of lignocellulosic biomass to ethanol uses 158 ℃ for the dilute-acid pretreatment reactor[11]; whereas, this study involves pretreatments from 140 ℃ to 160 ℃. Four control runs were performed at each temperature with dilute acid but without Lewis acids since it is possible for each reactor to provide different results due to differences in the heating and mixing systems. The experimental designed for this study was listed in Table 1. The table represents a total of 10 Lewis acid pretreatment experiments that were repeated for each of the 4 Lewis acids and 12 experiments for the control pretreatments. The pretreatments including Lewis acids at 150 ℃ were only performed at 5.5 mM as center points.

In order to clearly predict the variation was only from Lewis acids concentration on above mentioned yields, the sulfuric acid concentration and reaction time were kept constant (0.5% w.t., 10 min) for all 52 runs.

3 Results and discussion

3.1 Monomeric sugar yields during pretreatment

The result of the xylose analysis on all 52 samples was shown in Figure 1. Each xylose yield is the average of two repeated pretreatments. The control experiment yields are the average of four pretreatments. From the experimental data it is clearly evident that at 1 mM Lewis acid concentrations from 140 ℃ to 160 ℃, there was negligible difference in xylose monomeric yields. From these results it can be concluded that experiments were conducted precisely without any outliers. However, it is observed that at 10 mM concentration from 140 ℃ to 160 ℃ there is visible difference in xylose yields. The highest monomeric yield at 160 ℃ and 10 mM was observed for $FeCl_3$ followed by $La(OTf)_3$. The results are in agreement with recent study conducted by Liu et al.[21], as they observed the highest xylose yield when corn stover was pretreated with 0.1 M of $FeCl_3$. It was interesting to find at the same condition the $AlCl_3$ had lower xylose yield even though it is very strong

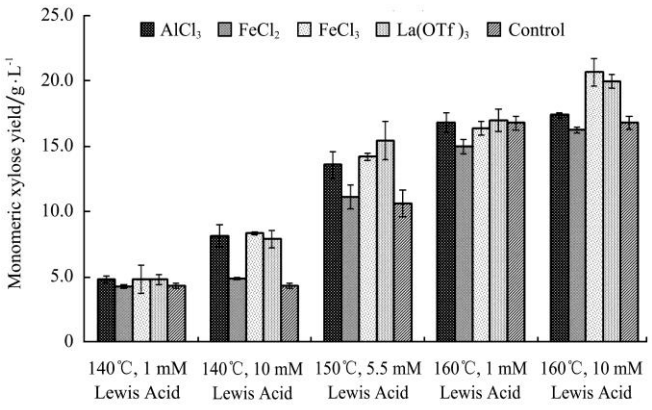


Figure 1 Monomeric xylose sugars yields in the liquid fraction of pretreated samples. Control experiments do not contain Lewis acids and are included for the sake of comparison

Lewis acid. Since, $AlCl_3$ was primarily degrading xylose into furfural as evident from Figure 3. From Figure 1 it can also be concluded that $FeCl_2$ Lewis acid was found to be insignificant effect in the xylose hydrolysis. The yields were almost similar to control samples.

3.2 Oligomeric sugar yields during pretreatment

The slurry liquor from the lower temperature pretreatment experiments consistently exhibited early peaks in HPLC which were typically indicative of carbohydrate oligomers due to the size exclusion effect of lead based carbohydrate columns used for HPLC[15]. As shown in the control pretreatments, there is a correlation between the pretreatment temperature and the concentration of oligomers (Figure 2). Oligomeric concentration was different between total xylose yield (from total sugar analysis) and monomeric xylose yield. In general, the higher temperature dilute acid pretreatments have much lower concentration of oligomeric sugars. Figure 2 also illustrates the correlation between the type and concentrations of Lewis acids and the relative concentration of oligomeric to total soluble xylose. The total oligomeric yield tended to be higher at low severity conditions, which could be due to the polymeric xylan depolymerizing to form oligomers more quickly at low temperatures than the depolymerization of the oligomers to form monomers[22]. An increase in the concentration of Lewis acids tends to reduce the percentage of oligomers. At 150℃ and at 5.5 mM Lewis acid concentration trend in the oligomeric sugar concentration was very similar to that at 160℃ and 10 mM. All the Lewis acids apart from $FeCl_2$ had lower oligomer concentration as compared to the control samples. In general, $FeCl_3$, $AlCl_3$, and $La(OTf)_3$ were effective in reducing the concentration of xylose in oligomeric forms. This effect is important due to two important factors: xylo-oligomers are difficult to break down with current enzymes which prevent their potential use in fermentation and xylo-oligomers have been shown to inhibit the action of cellulases on the cellulose portion of the pretreated biomass[23]. Furthermore, the latest NREL Process Design Report[11] utilizes a separate oligomer hydrolysis unit operation to further hydrolyze the oligosaccharides into monosaccharides. If the addition of a 10 mM of Lewis acids can reduce the concentration of total xylose present in oligomeric form in the slurry liquor after pretreatment it may be possible to remove this unit operation entirely. The reduction in oligomers may also lead to higher yields during enzymatic saccharification and fermentation.

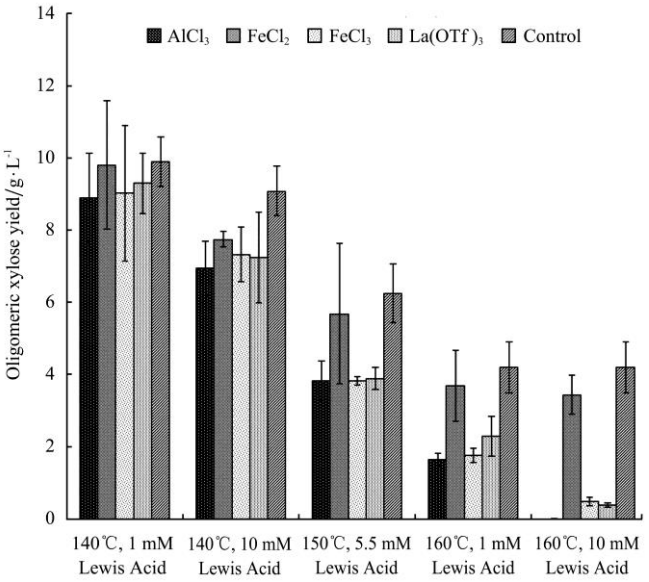


Figure 2 Xylo-oligomers relative to total soluble xylose in the slurry liquor after the dilute acid pretreatment of corn stover. Xylo-oligomers were quantified via an additional dilute acid hydrolysis of the slurry liquor.

3.3 Furfural formation during pretreatment

The major inhibitors found in the liquid fraction of the pretreated samples were furfural. In general, when a Brønsted acid such as H_2SO_4 is used as a catalyst, the dehydration of xylose to furfural formation follows one-step process as seen from Equation (2)[24]. However, Lewis acids such as $AlCl_3$ are used in a biphasic system as it follows a two-step process. Firstly, in the presence of Lewis acids xylose isomerizes to form xylulose and dehydration of xylulose in the presence of Brønsted acid yields furfural as evident from Equation (3)[25]. It was interesting to note that the concentration of furfural at 140℃ and 150℃ was found to be negligible (almost zero concentration). This was primarily due to limitation in the RID detector. Any concentration with a lower limit of 0.1 g/L was not detected. However, furfural was observed for all the samples that were treated at 160℃ irrespective of the

Lewis acid concentration. The overall concentration of furfural formation was higher for biomass samples pretreated with Lewis acid $AlCl_3$ at 10 mM concentration as evident from Figure 3. The furfural results follow the trend based on the evaluation done by Pearson on Hard and Soft Lewis acids[26]. According to the study, Al^{3+}, Fe^{3+}, and La^{3+} come under category of Hard Lewis acids. Hence, higher furfural yield was observed. However, Fe^{2+} comes under border line between Soft and Hard Lewis acids. It led lower furfural yield as evident from Figure 3.

$$\text{Xylose} \xrightarrow[-3H_2O]{\text{Bronsted acid}} \text{Furfural} \quad (2)$$

$$\text{Xylose} \xrightarrow{\text{Lewis Acid}} \text{Xylulose} \xrightarrow[-3H_2O]{\text{Bronsted acid}} \text{Furfural} \quad (3)$$

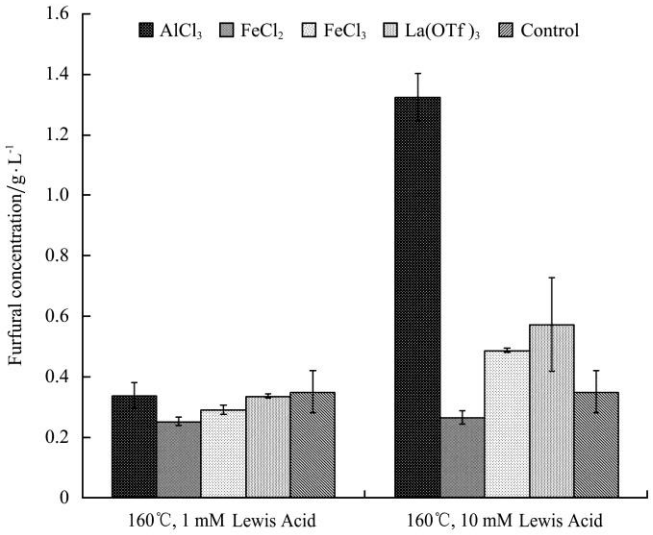


Figure 3 Concentration of furfural in the pretreated liquid hydrolyzate samples. Control experiments do not include Lewis acids and are included for comparison.

A similar trend was observed from glucose degradation into HMF[27] as evident from Figure 4. It was interesting to note that at 140 ℃, 1 mM concentration the HMF yield for $La(OTf)_3$ was very low. This average data point can easily be considered as experimental error or as an outlier. In addition, the concentration of HMF ranged from 0.15 g/L at 140 ℃ to a maximum of 0.6 g/L. The low HMF concentration was due to low severity conditions or the other possible reason is that HMF rehydrolyses in the presence of water to form formic and levulinic acid[28].

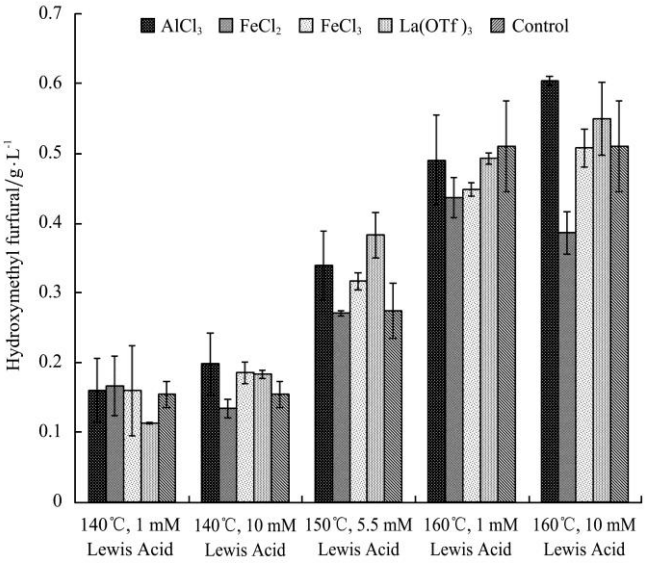


Figure 4 Concentration of HMF in the pretreated liquid hydrolyzate samples. Control experiments do not include Lewis acids and are included for comparison.

Moreover, the study conducted by Weil et al.[29] measured the maximum toxicity level of furfural on ethanol producing bacteria. The study shows that *Saccharomyces cerevisiae* bacteria can tolerate concentration of furfural in the range of 3-4 g/L during formation of bio-fuel from fermentable sugars. Since, the concentrations for furfural observed from the results were well below the tolerance limit of the bacteria, it can be concluded that addition of these Lewis acids as co-catalyst could be ideal during the pretreatment without any detrimental effects during enzymatic Saccharification and fermentation. These results were in agreement with experiments conducted by Kamireddy et al[30]. They studied the effects of three metal chlorides, $FeCl_3$, $CuCl_2$, and $AlCl_3$, without dilute sulfuric acids on corn stover. The results showed that higher Lewis acid concentration during pretreatment led to higher enzymatic digestibility. A similar results were also experimentally observed by Liu et al[21].

3.4 Enzymatic saccharification

The pretreated solid substrate mostly contains cellulose, lignin with trace of hemicellulose. The enzyme loading was based on the amount of cellulose content retained after pretreatment. Saccharification was performed primarily to evaluate whether the presence of these Lewis acids as co-catalyst had any adverse

effects in the cellulose digestibility. From Figure 5, it was clearly evident that there was no such unfavorable effect; in fact some Lewis acids had increased the yields slightly than control samples. The maximum glucose yields during enzymatic saccharification were observed for 10 mM $AlCl_3$ at 84% w.t. followed by $FeCl_3$ at 160 ℃ 81% w.t.

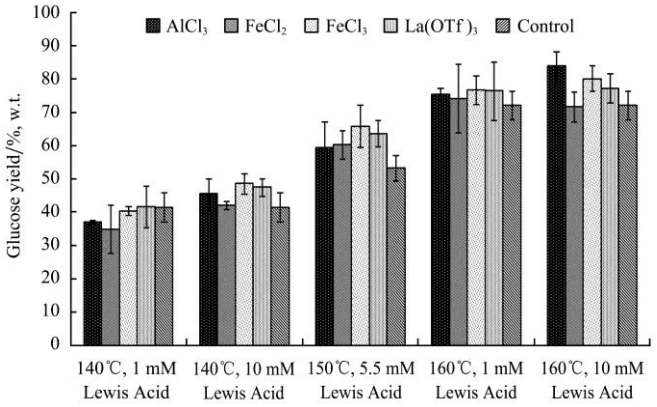
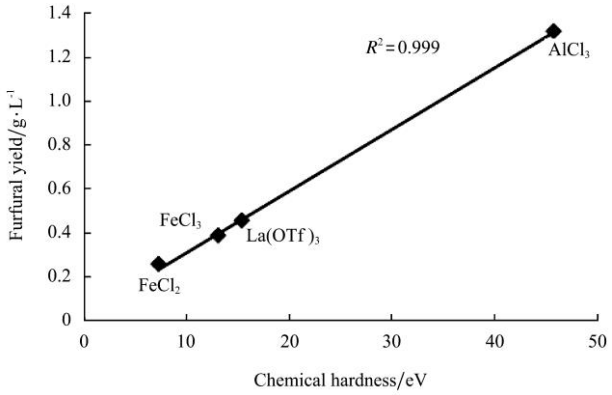


Figure 5 Yield of glucose during the enzymatic saccharification of the dilute acid pretreated solids co-catalyzed with Lewis acids

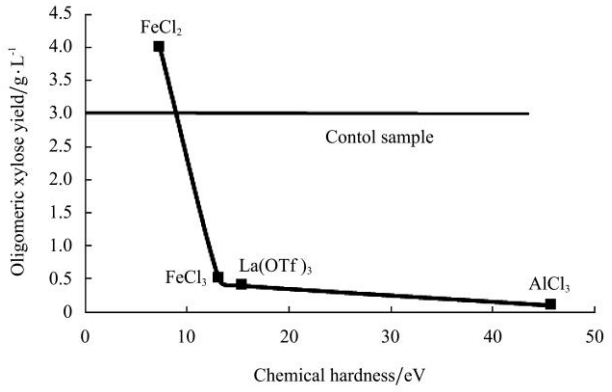
The presence of lignin generally has negative effect on the enzymatic hydrolysis yields. Since, enzymes that are adsorbed by lignin sites form lignin-enzyme complexes and considered as ineffective[16]. The increase in yield from control samples was mainly due to presence of (Al^{3+}, Fe^{3+}, La^{3+}) cations can reduce the lignin inhibition through formation of lignin-metal complexes. Hence, more active cellulose sites were accessible by the cellulase enzymes for hydrolysis. These results were in agreement with studies conducted by Liu et al[21]. However, for samples pretreated with $FeCl_2$ had almost similar yields as control samples (no significant increase was found). It was primarily due to presence of higher xylan content (data not shown) in the solid fraction for the biomass after the pretreatment. This was also evident from the lower concentration of xylose from Figure 1.

3.5 Hard-soft acid-base theory

Hard Lewis acids or bases are those that exhibit low polarizability and high electronegativity whereas soft acids and bases are more polarizable and have lower electro negativities[31]. The qualitative concept of chemical hardness can be quantified as shown in the previous study[31]. As, Al^{3+} exhibits the highest value of chemical hardness (45.8 eV) as compared to Fe^{2+} (7.3 eV) displayed a significantly higher yield of furfural at 160 ℃ and a 10 mM concentration as shown in Figure 6a. From a qualitative perspective there seems to be some interaction between the chemical hardness and the behavior of each Lewis acid during pretreatment. Figure 6a displays the furfural concentration at 10 mM Lewis acid runs at 160 ℃ versus the chemical hardness. A simple linear regression yields an excellent fit between the furfural concentration and the chemical hardness.



a. Furfural concentration



b. Oligomer reduction with Lewis acid co-catalyzed with dilute sulfuric acid at 160 ℃ and 10 mM Lewis acid loading

Figure 6 Effect of chemical hardness upon furfural yield and oligomeric xylose yield

As mentioned earlier in the section 3.2, there is also a correlation between the reduction in xylo-oligomers and the hardness of Lewis acids. In Figure 6b, plot indicates the concentration of xylose in oligomeric form versus the chemical hardness of the Lewis acids. It appears that hard Lewis acids ($AlCl_3$, $FeCl_3$, and $La(OTf)_3$) at 160 ℃ and 10 mM concentration had significant reduction in xylo-oligomer as compared border line Lewis acid ($FeCl_2$) and control samples. This trend was also significant at

lower temperatures pretreatments for hard Lewis acids as reduction in oligomers was clearly observed. From the data it is evident that hard Lewis acids were able to hydrolyze polymeric hemicellulose during pretreatment much more efficiently compared to control samples. These results are in agreement with the study conducted by Liu et al[21]. The addition of hard Lewis acids such as $AlCl_3$ led to high furfural concentration during the biomass pretreatment. The results were in agreement with the study conducted by Kamireddy et al[30]. However, detailed studies have to be conducted to investigate the interaction mechanisms between dilute Brønsted acids and Lewis acid co-catalysts during biomass pretreatment.

3.6 Effect of pH value

It can be assumed that the addition of Lewis acids especially hard Lewis acids would reduce the pH value of the solution further enhancing the monomeric xylose yields. However, the drop in pH for solution with and without Lewis acids was undetected prior to pretreatment. This was primarily due to very low concentration of Lewis acids (mM). However, after pretreatment the pH values were increased with increase in pretreatment temperature. It was due to cleavage of acetyl linkages of hemicellulose thus forming acetic acid in the pretreated hydrolyzate samples (data not shown)[16]. In addition, the study conducted by Peng et al.[32] was on the conversion of cellulose into levulinic acid. The study was performed based on the different metal chlorides under the same initial pH values of the reaction system and found that even at the same initial pH value, the yields of levulinic acid formed from rehydration of HMF were different with various metal chlorides[32]. From these results it can be concluded that type of metal chlorides played a major role in the hemicellulose hydrolysis into monosaccharaides rather than pH value of the solution.

4 Conclusions

Lewis acids can alter the results expected from the co-catalyzed dilute acid pretreatment and subsequent enzymatic saccharification. So-called hard Lewis acids tend to significantly reduce the abundance of oligo-saccharides and particularly xylo-oligomers. The chemical hardness of each Lewis acid has a good correlation with the production of furfural during pretreatment which is a well-known inhibitor during fermentation. The harder Lewis acids ($AlCl_3$) also tended to produce slightly more HMF during pretreatment versus the dilute acid only control pretreatments. The hard Lewis acids also tended to give higher yields of glucose during the enzymatic saccharification. Hence, it can be concluded that the addition of Lewis acids as co-catalysts, mainly $FeCl_3$, $AlCl_3$, in minute concentrations can lead to good fermentable sugar yields without any adverse effects. The addition of Lewis acids amount if optimized precisely there is scope for eliminating the secondary hydrolysis unit operation after pretreatment. Thus, bio-fuel process operation can be more economical.

Acknowledgments

This study was financially supported by National Renewable Energy Laboratory Subcontract No. AEV-0-40634-01 and North Dakota Experimental Program to Stimulate Competitive Research (EPSCoR).

[References]

[1] Dale B. Biofuels: Thinking clearly about the issues. Journal of Agricultural Food Chemistry, 2008; 56(11): 3885-3891.

[2] Kumar R, Wyman C E. Cellulase adsorption and relationship to features of corn stover solids produced by leading pretreatments. Biotechnology Bioenergy, 2009; 103(2): 252-267.

[3] Zheng Y, Pan Z L, Zhang R H. Overview of biomass pretreatment for cellulosic ethanol production. International Journal of Agriculture & Biological Engineering, 2009; 2(3): 51-68.

[4] Dien B S, Sarath G, Pedersen J F, Sattler S E, Chen H, Funnell-Harris D L, et al. Improved sugar conversion and ethanol yield for forage sorghum (Sorghum bicolor L. Moench) lines with reduced lignin contents. Bioenergy Resource, 2009; 2(3): 153-164.

[5] Mosier N S, Wyman C, Dale B. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology, 2005; 96(6): 673-686.

[6] Kootstra A M J, Beeftink H H. Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. Biochemical Engineering, 2009; 46(2): 126-131.

[7] Harmsen P, Huijgen W, Bermudez L, Bakker R. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Biosynergy, 2010; 1184(10): 1-53.

[8] Daniel J S, Farmer J, Newman M, McMillan J D. Dilute–Sulfuric acid pretreatment of corn stover in pilot scale reactor. Applied Biochemistry and Biotechnology, 2003; 105-108: 69-85.

[9] Modig T, Lidén G, Taherzadeh M J. Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. Journal of Biochemistry, 2002; 363(3): 769-776.

[10] Qing Q, Yang B, Wyman C E. Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresource Technology, 2010; 101(24): 9624-9630.

[11] Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A. Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: Dilute-acid pretreatment and enzymatic hydrolysis of corn stover. NREL Report, 2011; TP-5100-47764.

[12] Wei H, Donohoe B S, Vinzant T B, Ciesielski P N, Wang W, Gedvilas L M, et al. Elucidating the role of ferrous ion co-catalyst in enhancing dilute acid pretreatment of lignocellulosic biomass. Biotechnology for Biofuels, 2011; 4: 48-64.

[13] Weiss N D, Farmer J D, Schell D J. Impact of corn stover composition on hemicellulose conversion during dilute acid pretreatment and enzymatic cellulose digestibility of the pretreated solids. Bioresource Technology, 2010; 101(2): 674-678.

[14] Degenstein J C, Kamireddy S R, Tucker M P, Ji Y. Novel batch reactor for the dilute acid pretreatment of lignocellulosic feedstocks with improved heating and cooling kinetics. International Journal of Chemical Reactor Engineering, 2011; 9(1): 1-9.

[15] Scarlata C, Hyman D. Development and validation of a fast high pressure liquid chromatography method for the analysis of lignocellulosic biomass hydrolysis and fermentation products. American Journal of Chromatography, 2010; 1217(14): 2082-2087.

[16] Kamireddy R S, Schaefer C, Defrese M, Degenstein J C, Ji, Y. Pretreatment and enzymatic hydrolysis of sunflower hulls for fermentable sugar production. International Journal of Agricultural and Biological Engineering, 2012; 5(1): 62-70.

[17] Donkoh E, Degenstein J, Tucker M, Ji Y. Optimization of enzymatic hydrolysis of dilute acid pretreated sugar beet pulp using response surface design. Journal of Sugar Beet Research, 2012; 49(1&2): 26-37.

[18] Nguyen Q A. Tucker M P. Dilute acid/metal salt hydrolysis of lignocellulosics. Patent: 2002; US 6423145.

[19] Fringuelli F, Pizzo F, Vaccaro L. Lewis-acid catalyzed organic reactions in water. The case of AlCl3, TiCl4, and SnCl4 believed to be unusable in aqueous medium. The Journal of Organic Chemistry, 2001; 66(13): 4719-4722.

[20] Kobayashi S, Nagayama S, Busujima T. Lewis acid catalysts stable in water. Correlation between catalytic activity in water and hydrolysis constants and exchange rate constants for substitution of inner-sphere water ligands. Journal of the American Chemical Society, 1998; 120(32): 8287-8298.

[21] Liu L, Sun J S, Cai C Y, Wang S H, Pei H S, Zhang J S. Corn stover pretreatment by inorganic salts and its effects on hemicellulose and cellulose degradation. Bioresource Technology, 2009; 100(23): 5865-5871.

[22] Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. NREL Report, 2008; TP-510-42623.

[23] Qing Q, Wyman C E. Hydrolysis of different chain length xylooliogmers by cellulase and hemicellulase. Bioresource Technology, 2011; 102(2): 1359-1366.

[24] Choudhary V, Sandler S I, Vlachos D G. Conversion of xylose to furfural using Lewis and Brønsted acid catalysts in aqueous media. ACS Catalysis, 2012; 2(9): 2022-2028.

[25] Yang Y, Hu C W, Abu-Omar M M. Synthesis of furfural from xylose, xylan, and biomass using $AlCl_3$ 6 H_2O in biphasic media via xylose isomerization to xylulose. ChemSusChem, 2012; 5(2): 405-410.

[26] Pearson R. Hard and soft acids and bases. Journal of American Chemical Society, 1963; 85(22): 3533-3539.

[27] Zhao H, Holladay J E, Brown H, Zhang Z C. Metal chlorides in ionic liquid solvents convert sugars to 5-hydroxymethylfurfural. Science, 2007; 316(5831): 1597-600.

[28] Qi W, Zhang S P, Xu Q L, Ren Z W, Yan Y J. Degradation kinetics of xylose and glucose in hydrolyzate containing dilute sulfuric acid. The Chinese Journal of Process Engineering, 2008; 8(6): 1132-1137.

[29] Weil J R, Dien B, Bothast R, Hendrickson R, Mosier N S, Ladisch M R. Removal of fermentation inhibitors formed during pretreatment of biomass by polymeric adsorbents. Industrial Engineering and Chemistry Research, 2002; 41(24): 6132-6138.

[30] Kamireddy S R, Li J, Degenstein J, Tucker M, Ji Y. Effects and mechanism of metal chlorides salts on pretreatment and enzymatic digestibility of corn stover. Industrial Engineering and Chemistry Research, 2013; 52(5): 1775-1782.

[31] Parr R, Pearson R. Absolute hardness: Companion parameter to absolute electronegativity. Journal of American Chemical Society, 1983; 105(26): 7512-7516.

[32] Peng L C, Lin L, Zhang J H, Zhuang J P, Zhang B X, Gong Y. Catalytic conversion of cellulose to levulinic acid by metal chlorides. Molecules, 2010; 15(8): 5258-5272.