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The Use of High-Solids Loadings in Biomass Pretreatment – A Review

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Abstract

The use of high solids loadings (≥15% solids w/w) in the unit operations of lignocellulose conversion processes potentially offers many advantages over lower solids loadings, including increased sugar and ethanol concentrations and decreased production and capital costs. Since the term lignocellulosic materials refers to a wide range of feedstocks (agricultural and forestry residues, distillery by-products, and dedicated energy crops like grasses), the term "solids loading" here is defined by the amount of dry material that enters the process divided by the total mass of material and water added to the material. The goal of this paper is to provide a consolidated review of studies using a high solids pretreatment step in the conversion process. Included in this review is a brief discussion of the limitations such as the lack of available water for mass transfer processes, increased substrate viscosity and increased concentration of inhibitors produced affecting pretreatment as well as, descriptions and findings of pretreatment studies performed at high solids, the latest reactor designs developed for pretreatment at laboratory- and pilot-scales to address some of the limitations, and high solids pretreatment operations that have been scaled up and incorporated into demonstration facilities.

Keywords: high solids, lignocellulose conversion, pretreatment, pilot scale, high density

Introduction

The production of commodity chemicals, (such as ethanol) from starch or lignocellulose, has a narrow profit margin. Studies utilizing low solids loadings (≤5% solids) are numerous and helpful; however, improved efficiency has prompted new studies using high-solids loadings. Over the last few years, several studies have begun to investigate the effects of high solids loadings (≥15% solids) on different unit operations within the process stream (Hodge et al., 2008; Jorgensen et al., 2007b; Kristensen et al., 2009b; Lu et al., 2010; Zhang et al., 2010) as a means of improving the economics.

The main advantage of using high solids loadings over low and moderate solids loadings is improved efficiency. Because there is a greater amount of biomass available in the reaction, higher sugar concentrations can be produced, which leads to increased ethanol concentrations (Hodge et al., 2008; Roche et al., 2009). The conversion process is more environmentally friendly, as less water is consumed (Stickel et al., 2009; Um & Hanley, 2008) under certain processing conditions. It should be noted that the water absorption capacity is a function of the lignocellulosic material, and significant water can be brought into the process, just through the selection of a particular type of material. However, some conversion processes have been developed to reduce process water and waste water by recovering and recycling liquid streams (Mohagheghi & Schell, 2010; Stenberg et al., 1998). Capital and production costs are greatly reduced. Smaller reactors and equipment can be utilized for equivalent sugar and ethanol production. Energy usage for heating, cooling, mixing and ethanol distillation is reduced, which renders the overall conversion process more efficient on an energy basis.

Current technology has allowed the use of up to 30% solids content in the fermentation of starch, whereas only 15-20% solids in lignocellulose conversion has been handled at the pilot plant scale (Jorgensen, 2009; Kristensen et al., 2009b). Zhang et al. (2010) estimate that a solids loading of approximately 30% lignocellulose should translate to an ethanol yield of 5-10% (w/w). This yield is the minimum desired for the distillation process to be economical, as the energy requirement for distillation is significantly reduced for ethanol concentrations above 4% (Larsen et al., 2008). To achieve this minimum ethanol concentration, some studies show that at least 15% solids (dry matter) is required for enzymatic hydrolysis (Jorgensen et al., 2007b; Kristensen et al., 2009a), while others estimate that minimum to be about 20% (Larsen et al., 2008). Although data for high solids pretreatment and hydrolysis are limited, it has been suggested that the combination of a high solids pretreatment followed by high solids hydrolysis has great potential at improving the process economics by increasing sugar and ethanol yields while decreasing capital costs (Hodge et al., 2008; Roche et al., 2009) However, utilizing high solids loadings in this conversion process is still relatively new, and more research is required to overcome certain challenges, like high concentrations of inhibitors and equipment mass transfer limitations that are not as apparent at the low and moderate solids loadings.

The goal of this review is to provide a consolidated source of information in regards to the latest advances in pretreatment technologies for high solids operations. Following a brief discussion of limitations affecting pretreatments performed at high solids, various pretreatment studies performed with moderate and high solids loadings are detailed and the latest reactor designs that address some of these limitations are discussed. Lastly, pretreatment operations that are known to have been successful at the pilot scale are summarized.

Factors limiting high solids pretreatments

Conventional pretreatments developed at lower solids loadings (5-10% solids) have long been shown to facilitate higher conversion of biomass into usable sugars compared to biomass which was not pretreated (Carvalheiro et al., 2008; Chen et al., 2009; Dadi et al., 2006; Galbe & Zacchi, 2007; Schell et al., 1992; Schwald et al., 1989; Wyman et al., 2005a). Some pretreatments, like AFEX, have been developed to require very little water (as low as 10% has been reported) (Wyman et al., 2005b) and have been referred to as “dry” pretreatments. However, as more pretreatment options are investigated with increased solids loadings, several challenges become apparent. For example, as the concentration of solid material increases, little to no free water may be available in the reactor (Kristensen et al., 2009b), which can limit the effectiveness of the chosen pretreatment. Acturally, the type of biomass utilized can have a large effect on the amount of feedstock-associated water that enters the process, as well as on the way the solid and liquid phases interact. Water binds differently to the different fractions of lignocellulosic material. Hemicellulose tends to have a high water-holding capacity, while cellulose and lignin do not (Weber et al., 1993). Water plays an essential role in pretreatment reactions, aiding in chemical and enzymatic reactions, reducing the viscosity of the slurry by increasing the lubricity of the particles, providing a medium for solubilization of sugars and other compounds and for mass transfer by diffusion. Many of the limitations associated with pretreatments that were not initially developed to perform at high solids loadings appear to be correlated with the lack of available water, which warrants further study in order to minimize these effects.

High solids slurries tend to be very viscous with some being paste-like in nature (Jorgensen et al., 2007a; Knutsen & Liberatore, 2010). Pretreated corn stover at 20% insoluble solids can be formed into shapes that remain even after applied forces are removed (Stickel et al., 2009). However, particle shape and size have a significant impact on the viscosity of a slurry since these characteristics influence the particle networking and type of packing that takes place within the slurry (Ehrhardt et al., 2010; Szijarto et al., 2011; Viamajala et al., 2009). For example, fibrous particles from straw or corn stover can easily become entangled, creating a very complex network of particles, which interact very differently than more nonfibrous particles like wood chips and corn cobs. A reduction in particle size has been shown to reduce viscosity (Viamajala et al., 2009), although, size reduction may not be feasible in all cases due to the large energy requirement for milling or grinding (Miao et al., 2011). High viscosities are associated with challenges like mixing and material handling that must be addressed for high solids pretreatments to be as effective as possible (Jorgensen et al., 2007a; Roche et al., 2009). Energy demands increase as mixing becomes more difficult, which may counteract the benefits of using high solids loadings. Reactors suitable for effective pretreatment of these complex networks of lignocellulosic materials are imperative, and designs implemented to overcome these limitations are discussed in a later section. Material handling also becomes an issue because viscous materials are difficult to pump or pour, which may limit the pretreatments' applicability in a conventional continuous system. The size, shape and concentration of particles should be taken into consideration to keep viscosity from limiting the conversion process.

While pretreatments at high solids loadings may be attractive for producing higher sugar concentrations, there is a risk for also producing higher concentrations of hydrolysis and fermentation inhibitors (Jorgensen et al., 2007b). Figure 1 shows some of the inhibitors that may be formed during the pretreatment of lignocellulose. . It is well documented that dilute acid pretreatment leads to the production of degradation products like acetic acid, furfural, hydroxymethylfurfural (HMF) and phenolic compounds (Bjerre et al., 1996; Georgieva et al., 2008; Hodge et al., 2008; Vertes et al., 2010), which have been shown to inhibit the other downstream steps in the conversion process. In addition to the type and severity of the pretreatment, the composition of lignocellulosic material may also contribute to the variety of inhibitors produced. For example, the hemicellulose found in herbaceous biomass like agricultural residues is composed mainly of xylose, whereas in softwoods, the hemicellulose is composed of mainly mannose (Galbe & Zacchi, 2007). Several studies have recently shown that sugars resulting from the hydrolysis of hemicellulose, like xylose, xylan and xylooligomers, have a significant impact on the conversion rates and yields of cellulase and β-glucosidase enzymes (Kim et al., 2011; Qing et al., 2010; Ximenes et al., 2011b). Pretreating agricultural residues under acidic conditions can lead to increased xylose yields, which can inhibit cellulase and β-glucosidase activity if these xylan hydrolysis products are not removed. However, inhibitor production is not limited to dilute acid pretreatment. Alkaline pretreatments performed at room temperature can produce aromatic compounds like furans, phenols (Klinke et al., 2004), low molecular weight acids (Knill & Kennedy, 2003) and aldehyde compounds (Vertes et al., 2010). Ximenes et al. (2011a) and Kim et al. (2011) have reported a significant decrease in activity and even deactivation in some instances for cellulase and two types of β-glucosidase exposed to low concentrations (2-5 mg/mL) of phenolic compounds. The enzymes were especially sensitive to the polyphenolic compound tannic acid. Tannins can be found in almost any part of the plant, so these findings are applicable to many biomass feedstocks. Optimization of pretreatment conditions to minimize inhibitor production, with consideration of the specific type and severity of the pretreatment and type and concentration of the biomass feedstock is necessary, as the combination of all of these variables is important when developing effective and efficient conversion processes (Figure 2).

Pretreatments

The most important result of a pretreatment is that it enables maximum sugar yield following enzymatic hydrolysis and minimizes the loss of sugars and the formation of inhibitory products. Pretreatments facilitate the degradation of lignocellulose by modifying or removing lignin and/or hemicellulose, increasing the surface area or decreasing the particle size (Balat et al., 2008; Jorgensen et al., 2007a) so that cellulose is more accessible to enzymatic hydrolysis.

Numerous pretreatments have been developed, and each has its advantages and disadvantages, making it beneficial to tailor the pretreatment to the biomass source and desired end use. Table I shows the effects various pretreatments have on the different fractions of lignocellulosic material.

Acid pretreatments

Pretreatments utilizing acids, especially dilute acid pretreatment, are the most commonly used pretreatment (Ehrhardt et al., 2010; Lloyd & Wyman, 2005; Wyman et al., 2005a; Zhu et al., 2004). During acid pretreatment, hemicellulose hydrolyzes into its respective monosaccharides, while the lignin condenses and precipitates (Balat et al., 2008; Galbe & Zacchi, 2007; Hendriks & Zeeman, 2009). Dilute acid reagents like sulfuric and phosphoric acids at concentrations ≤4% are typically utilized at elevated temperatures (140-200ºC) for up to 1 hr (Galbe & Zacchi, 2007). Sulfur dioxide has also been used as an acid catalyst in conjunction with steam pretreatment (Chandra et al., 2007). While acid pretreatment is effective in the breakdown of lignocellulosic material, it can result in many degradation products, like furfural, HMF and acetic acid (Vertes et al., 2010), which can be inhibitory in downstream processes. Other disadvantages associated with acid pretreatment include the loss of some fermentable sugars due to degradation, high costs of reactor materials which are resistant to corrosion, and the additional cost of neutralizing the acid prior to downstream processing (Galbe & Zacchi, 2007; Hendriks & Zeeman, 2009; Mosier et al., 2005).

One of the earliest studies published regarding pretreatment at high solids loadings was one utilizing SO_2 at 33% solids loading (Wayman et al., 1987). Aspen and corn stover were pretreated for 30 min at 160ºC using 3% (w/w) SO_2 in a direct steam reactor. Solubilized hemicellulose sugar yields from aspen were $\geq$90% of the theoretical yield, with a significant reduction (25.5% to 5.6%) in soluble oligomer yield during pretreatment when compared to steam pretreatment without SO_2, which is a favorable result. The pretreatment of corn stover also resulted in solubilization of 79% of the hemicellulose sugars. Subsequent enzymatic hydrolysis and fermentation of aspen resulted in 91% and 73% theoretical glucose and ethanol yields, respectively, while corn stover resulted in 86.5% and 81% theoretical glucose and ethanol yields, respectively. One benefit of SO_2 over H_2SO_4 as an acid catalyst is that the pH is not lowered and the washing step between pretreatment and hydrolysis can be omitted without limiting enzymatic hydrolysis. SO_2 is also more compatible with stainless steel than H_2SO_4, and lignin may be better preserved, allowing for uses like heating and/or powering the conversion process or other higher value applications.

Another early pretreatment study utilizing high solids loadings selected dilute sulfuric acid as a catalyst (Schell et al., 1992). The pretreatment consisted of two steps. The first step was soaking corn stover at 10% solids loading for 24 hr. The second step involved applying steam followed by flash cooling the corn stover. Although exact solids loading was not given, the researchers estimated that it was between 20 and 30%. Under the pretreatment conditions tested, the xylan was reduced by nearly 50% at the lower severities to nearly 100% at the higher severities. The subsequent glucose yields from enzymatic hydrolysis increased with increasing pretreatment severity from approximately 55% to 96% yield with the exception of the highest severity (77% glucose yield). It is possible the production of degradation products from these pretreatment conditions (180ºC for 20 min) inhibited the enzymes digesting the corn stover. The authors also noted that optimization of pretreatment conditions for enzymatic hydrolysis may not optimize fermentation, since the presence of these degradation products (i.e. HMF, furfural) are toxic to fermentative yeasts. The entire process should be evaluated as one system rather than optimizing each unit operation individually.

Continued research resulted in studies of a pilot-scale system (1 ton/day) capable of continuously pretreating corn stover at 20% solids loading (Schell et al., 2003). A range of temperature, acid concentration and retention times were studied and compared by a value known as the combined severity factor (CSF). The severity factor was developed as a means for combining the temperature of a reaction with the time spent at that temperature into a single value (Overend & Chornet, 1987). It is used to rate processes as times and temperatures can be altered, while still maintaining a constant pretreatment severity. The CSF was further developed to include the pH at which the reaction takes place. It is used to facilitate the comparison among different pretreatment processes and conditions as it incorporates the pretreatment temperature, reaction time and the pH as follows:

$$\log R_o = \log\left(t \times e^{\left(\frac{T-100}{14.75}\right)}\right) - pH \qquad \text{Equation 1}$$

where t is the reaction time in min and T is the temperature in ºC (Galbe & Zacchi, 2007; Kabel et al., 2007). It was determined that the optimum xylose yield (~70%) occurred for pretreatment conditions with a CSF in the range of 1.4-1.7. As the CSF increased above this range, xylose yields decreased, which was most likely the result of the monosaccharides forming degradation products like furfural. While CSF is a means of comparison among different pretreatment conditions, it does not necessarily provide an indication of the pretreatment effectiveness. Only a slight positive relationship was observed between CSF and cellulose conversion.

A percolation reactor designed by Zhu et al. (2004) was evaluated using 25% solids loading and an acid flowrate of 10 mL/min . It was observed that the acid exiting the reactor within the first several minutes had a higher pH than when the acid entered the reactor. The researchers attributed this pH change to the buffering capacity of the corn stover at high solids loading. This same buffering capacity was also observed by Schell et al. (2003), where the main focus of the study was the production of xylose. While xylose yield increased with increasing time and temperature of pretreatment, the increased time also resulted in further dilution of the monosaccharides. Other monosaccharides (glucose, arabinose, galactose and mannose) were also detected in the eluent. Further testing would be required to optimize the process for maximum sugar production, whether it is for a single desired monosaccharide or a combination.

In a later study (Zhu et al., 2005), it was determined that the optimum pretreatment conditions for corn stover in the percolation reactor were 170ºC and 1.0% (w/w) acid applied at 10 mL/min. Mass balance closures accounted for ≥94% of the xylose and glucose monosaccharides, with nearly 100% glucan digestibility. Two observations arose from the biomass pretreatment that may warrant further investigation. (1) Due to the axial position of the reactor, the corn stover at the inlet experiences a reaction time nearly double that of the corn stover at the outlet. (2) The CSF changed over the length of the reactor because of changes in the buffering capacity of the corn stover. These two issues led to a non-uniform pretreatment of the corn stover that may have several implications in the overall process. The corn stover located nearer to the inlet of the reactor is exposed to acids at lower pH for prolonged periods of time, thus potentially resulting in an increased production of degradation products. Furthermore, the corn stover nearer to the outlet of the reactor may not be fully converted to fermentable sugars since the acid is buffered and the reaction time is shorter

Acidic pretreatments typically remove the hemicellulose fraction by hydrolyzing it into its monosaccharide components, which facilitates enzymatic hydrolysis of the remaining cellulose. However, it has been shown that as reaction time, temperature, acid concentration or a combination of these three is increased beyond a certain point, xylose yield in the pretreatment liquor decreases. This decrease in xylose yield is typically attributed to xylose decomposing into other degradation products. Lu et al. (2009) observed similar trends. They reported xylose yields increasing when they increased the acid concentration or increased the reaction time. At a 2% acid concentration, xylose yields decreased with increasing reaction times. Acetic acid, HMF and furfural production were observed, but the concentrations were below inhibitory levels for yeast fermentation.

While sulfuric acid is most commonly used in dilute acid pretreatments, other organic acids, like fumaric acid and maleic acid, have been tested (Kootstra et al., 2009). Kootstra et al. (2009) measured glucose and xylose yields after pretreating wheat straw at 20% and 30% solids loadings with sulfuric, maleic and fumaric acids. Maleic and fumaric acids do not promote the reactions that lead to sugar degradation products (i.e. furfural and HMF) that often result from pretreatment with sulfuric acid. An additional benefit of these two acids over sulfuric acid is that the quality of the by-product stream changes from excessive amounts of gypsum to fertilizer or feed components. The acid to wheat straw ratio used was 5.17% (w/w), which is slightly higher than acid concentrations typically used in dilute acid pretreatment. For a given set of pretreatment conditions, glucose yields varied by up to as much as 30 percentage points among the three acid pretreatments. The xylose yields decreased with increasing solids loadings for sulfuric and maleic acid but increased slightly for the fumaric acid pretreatment. Additionally, furfural production was more significant for the sulfuric acid pretreatment than the other two treatments, which was expected based on the reaction mechanisms of the different acids. While the overall results for maleic acid were promising, price is a limiting factor, since maleic acid can cost at least ten times that of sulfuric acid.

Although the number of studies using acid pretreatment in a high-solids environment is limited, there appears to be an emerging consensus for the optimal conditions to utilize in dilute acid pretreatment (Table II) to maximize glucose yields. Based on the conclusions of the high solids studies reviewed, at solids loadings ≥20%, an acid concentration of 1% (w/w) at ~180ºC with a reaction time ≤10 min resulted in optimal xylose yields from pretreatment and glucose yields from subsequent enzymatic hydrolysis (Lu et al., 2009; Schell et al., 2003; Schell et al., 1992; Zhu et al., 2004; Zhu et al., 2005).

Alkaline pretreatments

Lime and NaOH are common reagents used for alkaline pretreatments, which can be conducted over a wide range of operating conditions (Galbe & Zacchi, 2007; Hendriks & Zeeman, 2009; Jorgensen et al., 2007a; Mosier et al., 2005). Reaction time can vary from several minutes to days, while temperatures can range from ambient to 150ºC (Galbe & Zacchi, 2007; Jorgensen et al., 2007a). Alkaline pretreatment effectively increases the surface area by swelling the biomass particles and increasing carbohydrate accessibility to enzymes, while reducing the degree of polymerization (DP) and crystallinity of the cellulose fraction. The hemicellulose fraction can be partially hydrolyzed under strong alkaline conditions. The bonds between the lignin and carbohydrates are broken, and some lignin is solubilized (Balat et al., 2008; Galbe & Zacchi, 2007; Hendriks & Zeeman, 2009; Jorgensen et al., 2007a). Other advantages associated with this pretreatment over other pretreatments like dilute acid and AFEX are low cost, use of less caustic materials, and recoverable and recyclable reagents (Mosier et al., 2005). Alkaline pretreatments do not require specialized equipment, as the alkaline reagents typically used do not cause corrosion like dilute acids, and high pressures like those used in AFEX are not utilized. Drawbacks of alkaline pretreatments include a large number of inhibitors which can be produced at the harsher operating conditions (Hendriks & Zeeman, 2009), and the effectiveness of these methods can be decreased with feedstocks with high levels of lignin, like woody biomass (Balat et al., 2008; Galbe & Zacchi, 2007).

A study conducted by Cheng et al. (2010) compared the common reagents for alkaline pretreatment. For the lime pretreatment, a solids loading of 10% (w/w) and alkaline loadings of 0-10% were tested for reaction times of 1-3 hours at 95ºC. The NaOH pretreatments were performed on 20% (w/w) solids with 0-4% alkaline loadings for 1-3 hours at 55ºC. Delignification increased up to 27.0% and 23.1% for the lime and NaOH reagents, respectively, as reaction time and alkaline loading increased. The authors also reported an increase in enzymatic hydrolysis conversion with increasing alkaline loading, with a maximum glucose conversion of 48.5% and 39.2% for lime- and NaOH-pretreated solids, respectively. It should be noted that the solids were not washed between the pretreatment and enzymatic hydrolysis steps.

A washing step is often used in other pretreatment protocols, but it introduces another point where the biomass must be handled, resulting in loss of material. While washing the biomass post-pretreatment can remove inhibitors, it also removes any solubilized sugars, reducing the overall yield. However, a post-pretreatment washing step in this study did not significantly increase the glucose yield for the NaOH-pretreated solids. Even though the pretreatment conditions are not identical for the different reagents, the results have interesting implications. NaOH pretreatments are promising for high solids pretreatments because glucose yields were similar to the yields produced from the harsher conditions of the lime pretreatment and because NaOH does not require a washing step after pretreatment.

Hydrothermal pretreatments

Hydrothermal pretreatments utilize water at elevated temperatures to improve the conversion of lignocellulose. Several pretreatment technologies are included in this category, including steam, steam explosion and hydrothermolysis. Further details on each of these pretreatments are provided below.

Steam and steam explosion pretreatments offer short reaction times on the order of 1-5 min but also require high temperatures (160-240ºC) and pressures (~1-3.5 MPa) (Galbe & Zacchi, 2007; Jorgensen et al., 2007a). The high temperature of steam promotes the deacetylation of hemicellulose, resulting in acidic conditions that further catalyze the reaction (Alvira et al., 2010). These pretreatment conditions may produce degradation products from the cellulose and hemicellulose, while lignin is redistributed but not removed (Mosier et al., 2005). Temperature and pressure combinations should be carefully chosen to maximize accessibility for enzymes and minimize the degradation products, which can inhibit the enzymes and fermentative organisms in other downstream processes. Steam pretreatment has been proven to be effective on most types of lignocellulosic material, with the exception being softwoods. The hemicellulose fraction of softwoods contains few acetyl groups (Alvira et al., 2010). However, steam pretreatment is ideal if the desired end-products are fibers; feedstocks can be separated into individual fibers with minimal loss of material (Balat et al., 2008). Steam and steam explosion pretreatments are also advantageous because they increase pore size, allowing for better accessibility of the saccharides for hydrolysis, making this pretreatment a cost-effective option for agricultural residues since steam is the reagent (Jorgensen et al., 2007a). The high energy content of the steam makes these pretreatments appropriate for use with high solids, as the amount of water added to the process can be reduced.

Hydrothermolysis, also known as liquid hot water (LHW) pretreatment can be used to hydrolyze lignocellulosic material. Like steam pretreatment, liquid water at elevated temperatures and pressures (180-230ºC and 2.4-2.8 MPa) acts much like an acid, as the pH of the water at 220ºC is about 5.5 (Allen et al., 2001; Mosier et al., 2005). Acetic acid, produced from deacetylation of the hemicellulose, also enhances the acid-catalyzed reactions. Under these conditions, LHW removes a significant portion of lignin (Mosier et al., 2005). Hemicellulose is also hydrolyzed into soluble sugars. However, pressure (~2.5 MPa) must be applied to keep the water in the liquid phase at the temperatures used (Mosier et al., 2005), requiring specialized equipment. Hydrothermolysis produces minimal inhibitors as compared to steam pretreatment and requires limited neutralization since no additional chemicals are used, but the overall concentration of soluble products tends to be lower than other pretreatments because a high volume of water is typically used (Hendriks & Zeeman, 2009; Mosier et al., 2005). Based on the nature of this pretreatment, solids loading is limited to about 20%.

A study comparing steam and LHW pretreatments at high and moderate solids loadings was conducted by Allen et al (2001). However, a direct comparison is difficult to make, considering the steam pretreatment was performed at 50% (w/w) and 70% (w/w) corn fiber solids loadings, while the LHW pretreatment was performed at 10% (w/w) solids loadings, due to reactor volume limitations. This study determined that the reaction medium, steam or liquid water, directly impacted the solubility of the substrate, the capacity to recover C5 sugars and the downstream processes. For example, the LHW pretreatment resulted in 61% solubilization of the corn fiber, while steam pretreatment resulted in 44% and 37% solubilization for solids loadings of 50% and 70%, respectively. This trend of similar or decreasing yields for increasing solids loadings in pretreatment is not uncommon (Kootstra et al., 2009; Luterbacher et al., 2010). This same negative correlation was also reported for C5 and C6 sugar recoveries as the solids loadings increased. Much of the hemicellulose fraction either underwent a transformation and reorganization within the insoluble portion of the corn fiber or degraded beyond useful monosaccharides at the higher solids loadings, resulting in a loss in fermentable C5 sugars. The final ethanol yield from the LHW pretreatment liquor was not impacted by the loss of C5 sugars; however, the rate of ethanol production from the liquid fraction (as compared to the simultaneous saccharification and fermentation of the pretreated corn fiber) was severely limited, likely because of solubilized inhibitory products. The liquid fraction produced from the steam pretreatment resulted in a reduced final fermentation rate and yield; however, the cause of the lower rate and yield is unknown because the inhibitor concentrations were similar to those found in the liquid fraction of the LHW pretreatment.

Another study utilizing LWH pretreatment with high solids loadings was conducted by Kim et al. (2008), with a mixture of wet distillers’ grains (WDG) and thin stillage as the biomass source at 13% to 30% solids loading. The by-products of the distilling process are typically used for nutritional supplements in the livestock and poultry industries; however, the high energy value of the residual sugars and fibers make these materials attractive as a feedstock for the production of energy or other high-value products. The LHW pretreatment did not degrade glucan or produce degradation products. Only 2.9% of the total glucan was converted to glucose during the pretreatment process, and no sugar degradation products were detected, which is a favorable characteristic of a pretreatment. In addition to the high solids loading for the pretreatment process, the WDG and stillage mixture was subjected to high solids enzymatic hydrolysis. The researchers report an increase in the glucose and xylose yields as solids loading for enzymatic hydrolysis increases to 20%, but the yields decrease at 30% solids loading. While the percentage glucose yields are comparable between the 13% and 30% solids loadings, the xylose yield is nearly double for the 30% solids loading. This increase can be explained by the fact that additional enzymes (xylanase and feruloyl esterase) were added to the mixture, which strengthens the argument that optimal enzyme mixtures may be required to reach the full potential of the biomass.

The Integrated Biomass Utilization System (IBUS) Project resulted in a continuous hydrothermal pretreatment reactor and process that is capable of processing wheat straw up to 100 kg/hr (Petersen et al., 2009). This process uses high temperatures (185-205ºC) and short residence times (6-12 min) to maximize both glucan and xylan recovery. The current process produces two process streams: the liquid fraction containing soluble xylan oligomers and degradation products and the solid fraction containing cellulose, insoluble hemicellulose and lignin. The solid fraction exits the reactor at approximately 25-40% DM. All pretreatment conditions studied except for one (205ºC for 6 min) produced glucose recoveries ≥90%;

however, hemicellulose recoveries covered a wide range (60-90%). Lower hemicellulose recovery is most likely due to the increase in production of degradation products at the higher severity pretreatment conditions, which was confirmed with further study of the inhibitors produced from the pretreated wheat straw (Thomsen et al., 2009).

Other/combination pretreatments

Other pretreatments utilized in high solids studies do not fall into any one particular category, as some pretreatments combine multiple processes to selectively produce sugars. The results of these studies are presented below.

Biphasic CO_2-H_2O

Several pretreatment approaches utilize water with acid or base additions to initiate the breakdown of biomass. The biphasic CO_2-H_2O pretreatment offers many advantages by combining these two reagents in the pretreatment process. The supercritical points for water and CO_2 are 22.1 MPa at 373.9ºC and 7.4 MPa at 31.1ºC, respectively. Utilizing elevated temperatures and pressures, water remains in the liquid phase, acting much like a LHW pretreatment, and CO_2 is in its supercritical fluid phase. The addition of the CO_2 acts as an acid catalyst in the reaction (Luterbacher et al., 2010), while the CO_2 found in the supercritical phase has also been shown to have a swelling effect on biomass. Lastly, the reagents can be easily separated and reused, keeping costs low, as CO_2 is immiscible in water at atmospheric conditions (Kim & Hong, 2001; Luterbacher et al., 2010). However, there is some additional capital costs associated with equipment suitable for pressurized systems.

The study performed by Luterbacher et al. (2010) is the first to combine this biphasic CO_2-H_2O pretreatment with high solids loadings (40% w/w). It is also one of the highest solids loadings reported for any pretreatment process. This pretreatment resulted in glucose yields above 70% for hardwoods and above 80% for switchgrass and corn stover, which are within ten percentage points from yields reported in other studies utilizing other leading pretreatment technologies (Luterbacher et al., 2010). These yields make this a promising pretreatment option, especially with good results from high solids loadings and inexpensive chemical reagents. However, conditions should be optimized for different biomass feedstocks in order to limit the amount of degradation products produced in this process. Furfural and HMF were both produced in measurable quantities in this study. Not only are these products inhibitory to the downstream conversion processes, but the sugar yields are reduced when these products are formed.

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL)

SPORL is a recently developed, yet promising, process that combines a sulfite treatment of wood chips under acidic conditions with mechanical size reduction with disk refining (Zhu et al., 2011; Zhu et al., 2009). This technique was specifically intended for the pretreatment of softwoods, for which other existing pretreatment technologies have had limited success in enhancing enzymatic hydrolysis yields. Conditions have since been investigated to include pretreatment of hardwoods (Wang et al., 2009). The SPORL process is a modification of the sulfite pulping process, which has been practiced at the industrial level for more than a century. The modifications made allow for nearly complete hemicellulose removal with minimal lignin condensation and removal. Some glucose is hydrolyzed in the process, but it is recovered at a later step. This pretreatment can be carried out with equipment (pulp digester and mechanical disk refiner) typically used in the pulp and paper industry. The pretreatment liquor can also be prepared and recovered with existing techniques, reducing costs associated with chemical needs and cleaning waste streams.

Zhu et al. (2009) investigated the combination of a sulfite treatment with mechanical size reduction by disk refining to enhance enzymatic hydrolysis of softwoods. This study was the first to establish this novel pretreatment process. Pretreatment conditions of spruce chips (20% w/v) that produced optimal cellulose conversion during enzymatic hydrolysis (<90%) was treatment with 8-10% bisulfite and 1.8-3.7% sulfuric acid for 30 min at 180ºC. Nearly all hemicellulose was removed, which exposed the underlying cellulose fraction to enzymatic attack. Additionally, furfural and HMF were produced in minimal concentrations, about 1 and 5 mg/g untreated wood, respectively.

In a later study performed by Wang et al. (2009), the SPORL process was expanded to include conditions appropriate for pretreatment of hardwood. At 20% (w/v), a bisulfite charge of 4% for 30 min at 180ºC produced the highest glucose yield following enzymatic hydrolysis. Unlike the SPORL process for the softwoods, sulfuric acid was not necessary to maintain the acidic pH due to the high acetyl concentration of hardwoods. Several benefits were recognized by not having to supply additional acid to the reaction. SPORL, under these conditions, could avoid reactor corrosion and substrate neutralization for optimal enzymatic hydrolysis, as well as negligible production of inhibitory products like furfural and HMF. It is apparent from the results of these studies that the SPORL process is effective for the pretreatment of woody biomass, but further studies should be conducted to determine appropriate conditions prior to use with other lignocellulosic materials.

Ammonia fiber expansion (AFEX)

Ammonia fiber explosion (or expansion) techniques have, in general, been well investigated as a pretreatment option for lignocellulosic material (Galbe & Zacchi, 2007; Jorgensen et al., 2007a; Kumar & Wyman, 2009). AFEX is a promising pretreatment option because it is effective in situations with high solids content and the ammonia reagent can be recycled (Jorgensen et al., 2007a), which can help in the reduction of processing costs. This method has also been shown to be effective on corn stover and other agricultural residues (Balat et al., 2008). AFEX works by applying a pressure, which is released after a short reaction time to cause the "explosion" of the lignocellulosic components. Temperatures typically range from 70-100ºC, with pressures of ~2 MPa and relatively short reaction times (5-10 min) (Galbe & Zacchi, 2007; Kim et al., 2008). While the lignin and hemicellulose fractions are not removed, some lignin-carbohydrate bonds are broken, subsequently making the cellulose and the hemicellulose available for enzymatic hydrolysis (Jorgensen et al., 2007a). However, it has been reported that AFEX can lead to the production of some inhibitors such as furfural if the processing conditions are not optimized for the material being pretreated (Jorgensen et al., 2007a).

Kim et al. (2008) conducted a study using AFEX to pretreat distiller's dried grains and soluble (DDGS) at high solids loading (Table II). The ammonia was applied at 0.8 g/g biomass, and the reaction was performed at 70ºC for 5 min. This pretreatment significantly increased the rate at which the biomass was hydrolyzed as compared to untreated DDGS, and complete conversion was achieved by 72 hrs. In the study presented here, with the high solids loading and ammonia recovery, the process is essentially a dry process, meaning the solids enter the reactor dry and leave the reactor dry. This aspect has interesting implications for the overall conversion process. The biomass can more easily be mixed to a desired solids loading for enzymatic hydrolysis or SSF, including higher solids loadings, thus reducing the amount of water needed in these downstream conversion processes. However, more research is necessary prior to utilization of biomass in this manner.

Steam explosion combined with NaOH and H_2O_2

The advantages of using NaOH and steam explosion individually as pretreatments were previously outline in the Alkaline Pretreatments section and the Hydrothermal Pretreatments section, respectively.

The combination of the steam explosion with the alkaline peroxide process allowed for the removal of hemicellulose and lignin, respectively (Yang et al., 2010). The cellulose content of the corn stover was effectively increased from 37.5% in its raw state to 45.2% to 73.2% following steam explosion and alkaline peroxide pretreatment, when pretreatments were applied in that order (Table II). A fed-batch process was also incorporated into the conversion process to gradually increase solids loading in enzymatic hydrolysis from the initial 12% to 30% at completion. This modification allowed for easier handling and mixing of the bulk material, while maintaining the viscosity at workable levels. Reducing-sugar yields increased from 90 g/L to 220 g/L at 12% and 30% solids loading, respectively. The combination of treatments used effectively removed lignin and hemicellulose and improved sugar conversion downstream.

Reactor design for high solids pretreatment

Reactors suitable for low to moderate solids loadings can limit the conversion process at high solids loadings due to ineffective mixing, which can result in increased concentrations of localized inhibitors, poor heat and mass transfer and requiring excessive amounts of energy to operate. Other considerations that should be included in the reactor design are the types of biomass and the size of particles that will be treated (Jorgensen et al., 2007a). Some types of biomass, like straw and rice, contain silica that can cause wear on moving parts. Also, larger particle sizes are preferred so the ratio of energy consumed to energy produced is as small as possible, and the more particle size reduction needed, the more significant energy input needed.

Reactors specifically designed to handle high-solids loadings are being developed for research purposes and use at laboratory- and pilot-scales are reviewed below. The reader is encouraged to view the original articles for more detailed descriptions of the reactors.

One of the earliest reactors designed specifically for high solids pretreatment was proposed by Hsu et al. (1996) and with which they successfully pretreated biomass at a solids loading of 10-15% (w/w). The design is based on classic paddle-blender designs and consists of a custom-fabricated, 100 L horizontal shaft reactor intended for dilute acid pretreatment of biomass at high solids loading at the pilot-scale. The reactor was constructed of Carpenter 20 Cb-3 stainless steel to accommodate dilute sulfuric acid at elevated temperatures and pressures (approximately 175ºC and 1.1 MPa). The horizontal orientation is advantageous as it limits the amount of particle settling and dead mixing zones found in other types of reactors (Dasari et al., 2009), while the scraping action of the paddle design aids in maintaining a clear reactor surface ensuring maximum heat transfer from the reactor jacket to the slurry (Hsu et al., 1996). The horizontal orientation also takes advantage of free-fall mixing, reducing the effect viscosity has on mixing. Power input to operate the reactor can be reduced since lower paddle speeds can still provide adequate mixing as compared to a vertically oriented reactor.

Jorgensen et al. (2007b) reported using a reactor similar in design to Hsu et al. (1996). Their reactor was also placed in a horizontal orientation to utilize free-fall mixing. However, it is divided into five separate chambers with a total capacity of nearly 280 L. Each chamber is fitted with three paddles on a variable-speed, rotating shaft to aid in the mixing process. Although the solids loading for pretreatment was not reported, the wheat straw exiting the reactor was at 23-28% DM. Along with operating as a pretreatment reactor, it can double as a reactor for simultaneous saccharification and fermentation (SSF), so that the entire conversion process can be conducted within one reactor. This design is beneficial in that it reduces the overall capital costs by eliminating the need for multiple reactors.

A laboratory-scale percolation reactor was designed and tested by Zhu et al. (2004) for dilute acid pretreatment of corn stover. It was constructed using Monel tubing, since this material is resistant to corrosion by acid. The reactor can be operated at pressures approaching ~2 MPa and at solids loadings of 25% (v/v). The acid flows through a heating coil prior to entering the reactor for pretreatment at the desired temperature (160-180ºC), while the effluent is cooled by a heat exchanger at the reactor exit. The flow rate of the dilute acid through the biomass can be controlled in order to optimize the saccharide yields while minimizing the production of inhibitory degradation products. This flow-through design also eliminates the potential problems associated with mixing a complex network of particles. The percolation reactor described by Zhu et al. (2004) has the advantage of operating in semi-batch mode, which provides several benefits to the dilute acid pretreatment process including: (1) Sugar products are discharged throughout the reaction process. By allowing the dilute acid to flow through the biomass, the pretreatment liquor contains fewer degradation products while the sugar yields are increased; (2) Larger amounts of lignin can be removed in semi-batch mode than in batch mode, which enhances cellulose availability in downstream processes; and (3) A packed bed reactor allows higher solids to liquid ratios, which can lead to increased sugar yields. It is worth noting that these benefits are specific to dilute acid pretreatment. Further study using the percolation reactor would be necessary to determine if these benefits transfer to other pretreatment regimes.

Pilot-scale operations

Several pilot-scale operations have incorporated high solids pretreatments into their conversion processes for research and development purposes. Some of the leading operations are discussed in further detail here.

In 2004, a demonstration plant designed by SEKAB E-Technology began operation in Sweden (S. Wännström, personal communication). This facility is the largest of its kind in Sweden (300-400 L/d bioethanol production capacity) and continues to be used as a development plant for industrial technology with a focus on bioethanol and biochemicals. The plant is fully equipped with all process steps from intake of the raw materials to the distillation of the final products and is designed to be flexible so that various kinds of feedstocks, pretreatments and other process concepts can be utilized for process optimization. The pretreatment system operates in a continuous mode at 25-40% solids loading under pretreatment conditions specifically selected for the available feedstock. For example, prior to the dilute acid pretreatment, the biomass can be conditioned with steam and/or acid (typically H_2SO_4 or SO_2) should it be necessary. Optimized procedures have been developed at this facility for both forestry and agricultural feedstocks. To date, SEKAB's demonstration plant has accumulated over 30,000 hours of operation, several patents and extensive knowledge for the production of ethanol from lignocellulose.

DONG Energy located in Denmark has a semi-continuous counter-current reactor that is capable of processing 100-1000 kg/hr and utilizing various pretreatments and feedstocks (Jorgensen et al., 2007a). This pilot plant is designed to test different pretreatment methods, to operate with larger particles and to operate at solids loadings up to 50% DM. It also has two separate pretreatment facilities for research purposes. One line is for research and development for continuous mode operation (≤100 kg/hr capacity), while the other is for mechanical development and scale-up (≤1 tonne/hr capacity) (Larsen et al., 2008). In 2009, DONG Energy opened a demonstration-scale operation in Kalundborg, Denmark. At this facility operated by Inbicon (a subsidiary of DONG Energy), the hydrothermal pretreatment of wheat straw is conducted at 30-40% solids loading. The pilot-scale facility is still used to optimize the process employed at the demonstration-scale facility.

The National Renewable Energy Laboratory (NREL) in the United States has a pilot-scale pretreatment reactor that operates at high solids loadings and has been the source of pretreated biomass for many high solids studies (Roche et al., 2009; Schell et al., 2003; Schell et al., 1992). It is used for continuous, dilute acid pretreatment of ≤30% solids loadings. Schell et al. (2003) provide a detailed description of the process. In August 2010, NREL completed the first phase in its Integrated Biorefinery Research Facility (IBRF). This expansion provides space for new pilot-scale biomass conversion equipment, including a continuous 1 ton/day horizontal pressure pretreatment reactor. This new facility will continue to be used as a research and development facility, studying various feedstocks and pretreatment options.

Direction of Future Work

In order to fully realize the advantages provided by pretreatment at high solids loadings, several issues must be addressed. The efficiency and effectiveness of a pretreatment process not only depends on the pretreatment conditions, but also on the type of biomass entering the pretreatment process. The pretreatment type and severity must be considered in combination with the biomass type and concentration to produce the most accessible and highest yielding saccharides while limiting the inhibitors entering other downstream steps in the conversion process. Other factors to consider during pretreatment optimization is the cost of biomass, reagents and any specialized equipment and the best use of any potential by-products produced in the process. Additionally, reactor systems robust enough to withstand a range of pretreatment conditions (temperature, pressure, reagent concentrations) and biomass properties (concentration, particle size, composition) are needed, especially for large scale production.

Conclusions

The feasibility of lignocellulosic conversion would greatly improve if high solids loadings could be used successfully in all the various unit operations. Increased sugar and ethanol yields combined with decreased capital and production costs and decreased water and power use contribute to a more efficient process compared to the conventional conversion process. As the benefits of utilizing high solids loadings in the lignocellulosic conversion process are realized, so too are the limitations. Issues associated with the lack of free water, the high viscosities and the increased production of inhibitors must be overcome in order to achieve economically viable sugar and ethanol yields. Researchers are tackling these problems on two fronts: reactor design and pretreatment optimization. Horizontal paddle reactors and percolation reactors have both been shown to be possible alternatives to standard reactor designs when it comes to high solids. The choice of pretreatment can also affect the effectiveness of the overall conversion process. The effort in optimizing these various pretreatment options for high solids is evident by the many studies discussed in this paper, but many questions still require answers before the full power of utilizing high solids is recognized.

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References

Allen, S.G., Schulman, D., Lichwa, J., Antal, M.J., Laser, M., Lynd, L.R. 2001. A comparison between hot liquid water and steam fractionation of corn fiber. *Industrial & Engineering Chemistry Research*, **40**(13), 2934-2941.

Alvira, P., Tomas-Pejo, E., Ballesteros, M., Negro, M.J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, **101**(13), 4851-4861.

Balat, M., Balat, H., Oz, C. 2008. Progress in bioethanol processing. *Progress in Energy and Combustion Science*, **34**(5), 551-573.

Bjerre, A.B., Olesen, A.B., Fernqvist, T., Ploger, A., Schmidt, A.S. 1996. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. *Biotechnology and Bioengineering*, **49**(5), 568-577.

Carvalheiro, F., Duarte, L.C., Girio, F.M. 2008. Hemicellulose biorefineries: A review on biomass pretreatments. *Journal of Scientific & Industrial Research*, **67**(11), 849-864.

Chandra, R.P., Bura, R., Mabee, W.E., Berlin, A., Pan, X., Saddler, J.N. 2007. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? in: *Biofuels*, Vol. 108, pp. 67-93.

Chen, M., Zhao, J., Xia, L.M. 2009. Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. *Biomass & Bioenergy*, **33**(10), 1381-1385.

Cheng, Y.S., Zheng, Y., Yu, C.W., Dooley, T.M., Jenkins, B.M., VanderGheynst, J.S. 2010. Evaluation of high solids alkaline pretreatment of rice straw. *Applied Biochemistry and Biotechnology*, **162**(6), 1768-1784.

Dadi, A.P., Varanasi, S., Schall, C.A. 2006. Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. *Biotechnology and Bioengineering*, **95**(5), 904-910.

Dasari, R.K., Dunaway, K., Berson, R.E. 2009. A scraped surface bioreactor for enzymatic saccharification of pretreated corn stover slurries. *Energy & Fuels*, **23**(1), 492-497.

Ehrhardt, M.R., Monz, T.O., Root, T.W., Connelly, R.K., Scott, C.T., Klingenberg, D.J. 2010. Rheology of dilute acid hydrolyzed corn stover at high solids concentration. *Applied Biochemistry and Biotechnology*, **160**(4), 1102-1115.

Galbe, M., Zacchi, G. 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. in: *Biofuels*, Vol. 108, Springer-Verlag Berlin. Berlin, pp. 41-65.

Georgieva, T.I., Hou, X.R., Hilstrom, T., Ahring, B.K. 2008. Enzymatic hydrolysis and ethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. *Applied Biochemistry and Biotechnology*, **148**(1-3), 35-44.

Hendriks, A., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, **100**(1), 10-18.

Hodge, D.B., Karim, M.N., Schell, D.J., McMillan, J.D. 2008. Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. *Bioresource Technology*, **99**(18), 8940-8948.

Hsu, T.A., Himmel, M., Schell, D., Farmer, J., Berggren, M. 1996. Design and initial operation of a high-solids, pilot-scale reactor for dilute-acid pretreatment of lignocellulosic biomass. *Applied Biochemistry and Biotechnology*, **57-8**, 3-18.

Jorgensen, H. 2009. Effect of nutrients on fermentation of pretreated wheat straw at very high dry matter content by *Saccharomyces cerevisiae*. *Applied Biochemistry and Biotechnology*, **153**(1-2), 44-57.

Jorgensen, H., Kristensen, J.B., Felby, C. 2007a. Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities. *Biofuels Bioproducts & Biorefining-Biofpr*, **1**(2), 119-134.

Jorgensen, H., Vibe-Pedersen, J., Larsen, J., Felby, C. 2007b. Liquefaction of lignocellulose at high-solids concentrations. *Biotechnology and Bioengineering*, **96**(5), 862-870.

Kabel, M.A., Bos, G., Zeevalking, J., Voragen, A.G.J., Schols, H.A. 2007. Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. *Bioresource Technology*, **98**(10), 2034-2042.

Kim, K.H., Hong, J. 2001. Supercritical CO_2 pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. *Bioresource Technology*, **77**(2), 139-144.

Kim, Y., Hendrickson, R., Mosier, N.S., Ladisch, M.R., Bals, B., Balan, V., Dale, B.E. 2008. Enzyme hydrolysis and ethanol fermentation of liquid hot water and AFEX pretreated distillers' grains at high-solids loadings. *Bioresource Technology*, **99**(12), 5206-5215.

Kim, Y., Ximenes, E., Mosier, N.S., Ladisch, M.R. 2011. Soluble inhibitors/deactivators of cellulase enzymes from lignocellulosic biomass. *Enzyme and Microbial Technology*, **48**(4-5), 408-415.

Klinke, H.B., Thomsen, A.B., Ahring, B.K. 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, **66**(1), 10-26.

Knill, C.J., Kennedy, J.F. 2003. Degradation of cellulose under alkaline conditions. *Carbohydrate Polymers*, **51**(3), 281-300.

Knutsen, J.S., Liberatore, M.W. 2010. Rheology modification and enzyme kinetics of high-solids cellulosic slurries: An economic analysis. *Energy & Fuels*, **24**, 6506-6512.

Kootstra, A.M.J., Beeftink, H.H., Scott, E.L., Sanders, J.P.M. 2009. Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. *Biochemical Engineering Journal*, **46**(2), 126-131.

Kristensen, J.B., Felby, C., Jorgensen, H. 2009a. Determining yields in high solids enzymatic hydrolysis of biomass. *Applied Biochemistry and Biotechnology*, **156**(1-3), 557-562.

Kristensen, J.B., Felby, C., Jorgensen, H. 2009b. Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. *Biotechnology for Biofuels*, **2**.

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

Larsen, J., Petersen, M.O., Thirup, L., Li, H.W., Iversen, F.K. 2008. The IBUS process - Lignocellulosic bioethanol close to a commercial reality. *Chemical Engineering & Technology*, **31**(5), 765-772.

Lloyd, T.A., Wyman, C.E. 2005. Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids. *Bioresource Technology*, **96**(18), 1967-1977.

Lu, X.B., Zhang, Y.M., Angelidaki, I. 2009. Optimization of H_2SO_4-catalyzed hydrothermal pretreatment of rapeseed straw for bioconversion to ethanol: Focusing on pretreatment at high solids content. *Bioresource Technology*, **100**(12), 3048-3053.

Lu, Y.F., Wang, Y.H., Xu, G.Q., Chu, J., Zhuang, Y.P., Zhang, S.L. 2010. Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. *Applied Biochemistry and Biotechnology*, **160**(2), 360-369.

Luterbacher, J.S., Tester, J.W., Walker, L.P. 2010. High-solids biphasic CO_2-H_2O pretreatment of lignocellulosic biomass. *Biotechnology and Bioengineering*, **107**(3), 451-460.

Mohagheghi, A., Schell, D.J. 2010. Impact of Recycling Stillage on Conversion of Dilute Sulfuric Acid Pretreated Corn Stover to Ethanol. *Biotechnology and Bioengineering*, **105**(5), 992-996.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, **96**(6), 673-686.

Overend, R.P., Chornet, E. 1987. FRACTIONATION OF LIGNOCELLULOSICS BY STEAM-AQUEOUS PRETREATMENTS. *Philosophical Transactions of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences*, **321**(1561), 523-536.

Petersen, M.O., Larsen, J., Thomsen, M.H. 2009. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. *Biomass & Bioenergy*, **33**(5), 834-840.

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

Roche, C.M., Dibble, C.J., Knutsen, J.S., Stickel, J.J., Liberatore, M.W. 2009. Particle concentration and yield stress of biomass slurries during enzymatic hydrolysis at high-solids loadings. *Biotechnology and Bioengineering*, **104**(2), 290-300.

Schell, D.J., Farmer, J., Newman, M., McMillan, J.D. 2003. Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor - Investigation of yields, kinetics, and enzymatic digestibilities of solids. *Applied Biochemistry and Biotechnology*, **105**, 69-85.

Schell, D.J., Walter, P.J., Johnson, D.K. 1992. Dilute sulfuric acid pretreatment of corn stover at high solids concentrations - Scientific note. *Applied Biochemistry and Biotechnology*, **34-5**, 659-665.

Schwald, W., Breuil, C., Brownell, H.H., Chan, M., Saddler, J.N. 1989. Assessment of pretreatment conditions to obtain fast complete hydrolysis on high substrate concentrations. *Applied Biochemistry and Biotechnology*, **20-1**, 29-44.

Stenberg, K., Tengborg, C., Galbe, M., Zacchi, G., Palmqvist, E., Hahn-Hagerdal, B. 1998. Recycling of process streams in ethanol production from softwoods based on enzymatic hydrolysis. *Applied Biochemistry and Biotechnology*, **70-2**, 697-708.

Stickel, J.J., Knutsen, J.S., Liberatore, M.W., Luu, W., Bousfield, D.W., Klingenberg, D.J., Scott, C.T., Root, T.W., Ehrhardt, M.R., Monz, T.O. 2009. Rheology measurements of a biomass slurry: An inter-laboratory study. *Rheologica Acta*, **48**(9), 1005-1015.

Szijarto, N., Siika-aho, M., Sontag-Strohm, T., Viikari, L. 2011. Liquefaction of hydrothermally pretreated wheat straw at high-solids content by purified *Trichoderma* enzymes. *Bioresource Technology*, **102**(2), 1968-1974.

Thomsen, M.H., Thygesen, A., Thomsen, A.B. 2009. Identification and characterization of fermentation inhibitors formed during hydrothermal treatment and following SSF of wheat straw. *Applied Microbiology and Biotechnology*, **83**(3), 447-455.

Um, B.H., Hanley, T.R. 2008. A comparison of simple rheological parameters and simulation data for *Zymomonas mobilis* fermentation broths with high substrate loading in a 3-L bioreactor. *Applied Biochemistry and Biotechnology*, **145**(1-3), 29-38.

Vertes, A.A., Qureshi, N., Blaschek, H.P., Yukawa, H. 2010. *Biomass to biofuels: Strategies for global industries*. John Wiley and Sons, Ltd.

Viamajala, S., McMillan, J.D., Schell, D.J., Elander, R.T. 2009. Rheology of corn stover slurries at high solids concentrations - Effects of saccharification and particle size. *Bioresource Technology*, **100**(2), 925-934.

Wang, G.S., Pan, X.J., Zhu, J.Y., Gleisner, R., Rockwood, D. 2009. Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) for Robust Enzymatic Saccharification of Hardwoods. *Biotechnology Progress*, **25**(4), 1086-1093.

Wayman, M., Seagrave, C., Parekh, S.R. 1987. Ethanol fermentation by *Pichia-stipitis* of combined pentose and hexose sugars from lignocellulosics prehydrolyzed by SO_2 and enzymatically saccharified. *Process Biochemistry*, **22**(2), 55-59.

Weber, C.W., Kohlhepp, E.A., Idouraine, A., Ochoa, L.J. 1993. BINDING-CAPACITY OF 18 FIBER SOURCES FOR CALCIUM. *Journal of Agricultural and Food Chemistry*, **41**(11), 1931-1935.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y. 2005a. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. *Bioresource Technology*, **96**(18), 2026-2032.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y. 2005b. Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology*, **96**(18), 1959-1966.

Ximenes, E., Kim, Y., Mosier, N., Dien, B., Ladisch, M. 2011a. Deactivation of cellulases by phenols. *Enzyme and Microbial Technology*, **48**(1), 54-60.

Ximenes, E., Kim, Y., Mosier, N., Dien, B., Ladisch, M. 2011b. Inhibition of cellulases by phenols. *Enzyme and Microbial Technology*, **46**(3-4), 170-176.

Yang, M.H., Li, W.L., Liu, B.B., Li, Q., Xing, J.M. 2010. High-concentration sugars production from corn stover based on combined pretreatments and fed-batch process. *Bioresource Technology*, **101**(13), 4884-4888.

Zhang, J., Chu, D.Q., Huang, J., Yu, Z.C., Dai, G.C., Bao, J. 2010. Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor. *Biotechnology and Bioengineering*, **105**(4), 718-728.

Zhu, J.Y., Gleisner, R., Scott, C., Luo, X.L., Tian, S. 2011. High titer ethanol production from simultaneous enzymatic saccharification and fermentation of aspen at high solids: A comparison between SPORL and dilute acid pretreatments. *Bioresource Technology*, **102**(19), 8921-8929.

Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R. 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresource Technology*, **100**(8), 2411-2418.

Zhu, Y.M., Lee, Y.Y., Elander, R.T. 2004. Dilute-acid pretreatment of corn stover using a high-solids percolation reactor. *Applied Biochemistry and Biotechnology*, **117**(2), 103-114.

Zhu, Y.M., Lee, Y.Y., Elander, R.T. 2005. Optimization of dilute-acid pretreatment of corn stover using a high-solids percolation reactor. *Applied Biochemistry and Biotechnology*, **121**, 1045-1054.

Table I. Effects of various pretreatment methods on lignocellulosic material.

Pretreatment	Cellulose	Hemicellulose	Lignin	Other Effects
Dilute Acid	Very little solubilization	High solubilization	Condensation and precipitation	--
Liquid Hot Water (LHW)	Very little solubilization	High solubilization	Delignification	--
Steam Explosion	Slight degradation	Slight degradation	Redistribution	Increase in pore size
Biphasic CO_2-H_2O	Very little solubilization	High solubilization	--	Increase in surface area
SPORL	Slight degradation	Nearly complete solubilization	Partial delignification and sulfonation	Reduction in particle size
Alkaline	Reduction in DP and crystallinity	Partial hydrolysis	Some solubilization	Increase in surface area
AFEX	--	Disruption of bonds with lignin	Disruption of bonds with carbohydrates	--

DP=degree of polymerization

Table II. Conditions of optimal sugar yields from pretreatments utilizing high solids loadings.

Pretreatment	Substrate	Solids Loading[a]	Residence Time	Temperature & Pressure	Other Conditions	% Sugar Yield[b] Glucose	Xylose	Ref.
Acidic Pretreatments:								
SO_2 + Steam	Hardwood	33%	30 min	160ºC, 0.5 MPa	3% (w/w) SO_2	91.2%	91.6%	(Wayman et al., 1987)
SO_2 + Steam	Corn stover	33%	30 min	160ºC, 0.5 MPa	3% (w/w) SO_2	86.5%	79.0%	(Wayman et al., 1987)
Steam	Corn fiber	70%	2 min	215ºC	--	87%	40%	(Allen et al., 2001)
Dilute Acid + Steam	Corn stover	20-30% [c]	10 min	180ºC	--	98%	NR[d]	(Schell et al., 1992)
Dilute Acid	Corn stover	20%	6.2 min	179ºC	1.16% (w/w) acid	87%	70%	(Schell et al., 2003)
Dilute Acid	Corn stover	25% (v/v)	3 min	180ºC	1% (w/w) acid, 10 mL/min	NR	73%	(Zhu et al., 2004)
Dilute Acid	Corn stover	25% (v/v)		170ºC	1% (w/w) acid	98.7%	94%	(Zhu et al., 2005)
Organic Acids	Wheat straw	20%	30 min	150ºC	5.17% (w/w) H_2SO_4	>90%	80%	(Kootstra et al., 2009)
Liquid Hot Water (LHW)	Corn fiber	10%	2 min	215ºC	--	93%	62%	(Allen et al., 2001)
LHW	WDG	20% (w/v)	20 min	160ºC	--	83%	50%	(Kim et al., 2008)
Hydrothermal	Wheat straw	25-40% [e]	6-12 min	195ºC	--	94%	70%	(Petersen et al., 2009)
Acid-Catalyzed Hydrothermal	Rapeseed straw	20%	10 min	180ºC	1% (w/w) acid	63.17%	75.12%	(Lu et al., 2009)

Biphasic CO_2-H_2O	Corn stover	20%	1 hr	160ºC, 20 MPa	--	85%	10%	(Luterbacher et al., 2010)
Biphasic CO_2-H_2O	Switchgrass	20%	1 hr	160ºC, 20 MPa	--	81%	13%	(Luterbacher et al., 2010)
Biphasic CO_2-H_2O	Hardwood	40%	1 hr	170ºC, 20 MPa	--	73%	14%	(Luterbacher et al., 2010)
SPORL	Softwoods	20% (w/v)	30 min	180ºC	8-10% (w/w) bisulfate + 1.8-3.7% (w/w) sulfuric acid	90%	76%	(Zhu et al., 2009)
SPORL	Hardwoods	20% (w/v)	30 min	180ºC	4% (w/w) sodium bisulfite	89%	NR	(Wang et al., 2009)
Basic Pretreatments:								
AFEX	DDGS	55%	5 min	70ºC	--	68%	12.2%	(Kim et al., 2008)
NaOH	Rice straw	20%	3 hr		4% (w/w) NaOH	39.2%	NR	(Cheng et al., 2010)
Steam Explosion with NaOH and H_2O_2	Corn stover	10%	24 hr	Room temperature	--	60%	NR	(Yang et al., 2010)

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[a] Solids loading is indicated in (w/w) unless otherwise noted

[b] Sugar yields are yields resulting from pretreatment and/or enzymatic hydrolysis

[c] Solids concentration following 24 hr soaking in 1% (w/w) sulfuric acid at 10% solids loading

[d] Not reported

[e] Concentration of dry matter exiting continuous reactor

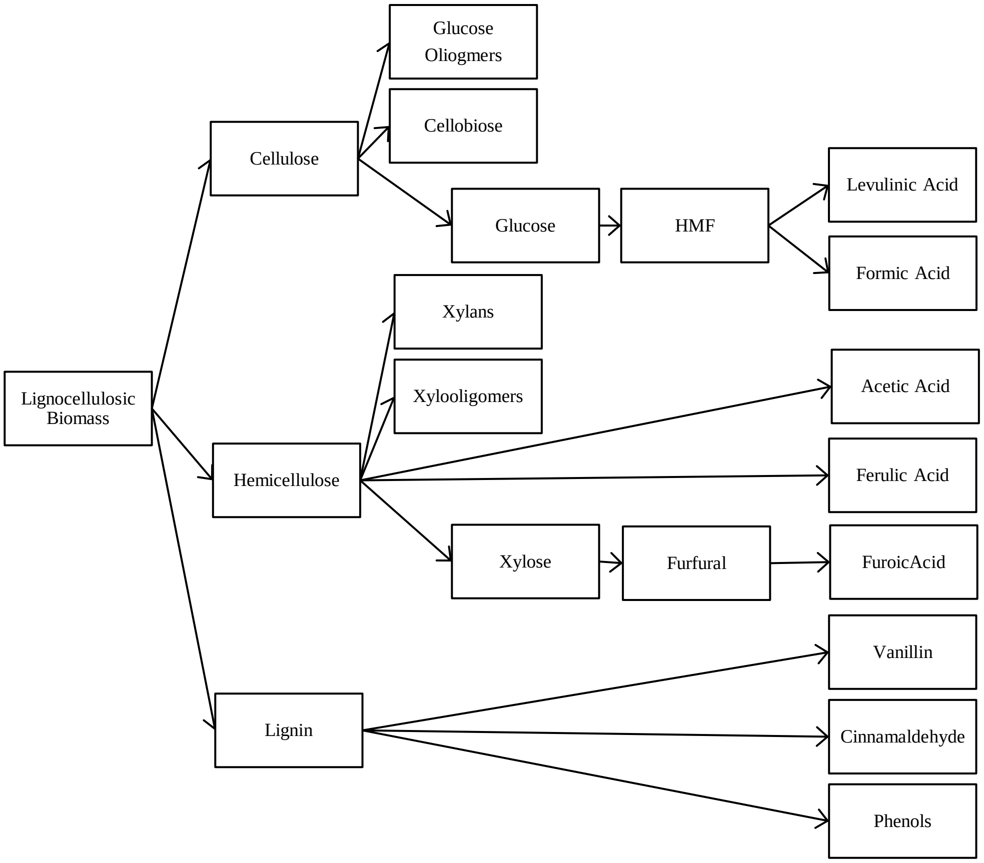

Figure 1. Schematic showing some of the products and potential inhibitors formed from the cellulose, hemicellulose and lignin fractions of lignocellulosic biomass during pretreatment.

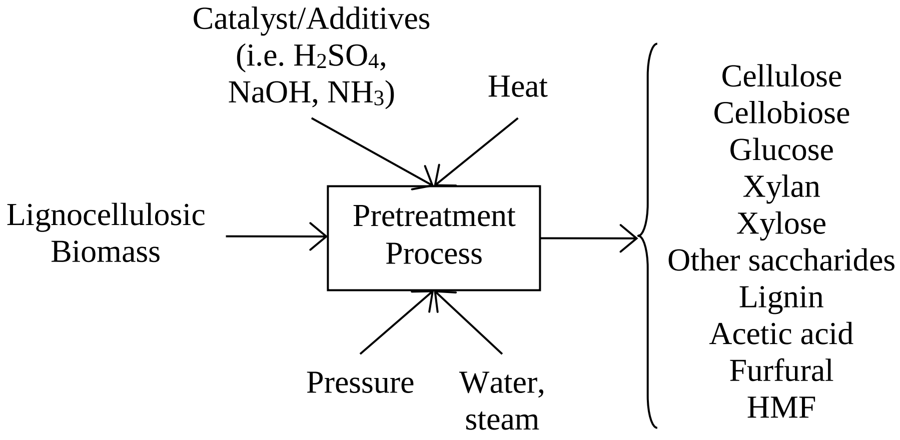

Figure 2. Schematic of a general pretreatment process.