Application of cellulase and hemicellulase to pure xylan, pure cellulose, and switchgrass solids from leading pretreatments

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1. Introduction

Sustainable alternatives for transportation fuels are needed to replace depleting petroleum-based options and address global climate change, and ethanol from cellulosic biomass has been highlighted as one of the most promising technologies in terms of technical and economical feasibilities (Wyman, 1994). A wide range of lignocellulosic biomass materials have been considered, including agricultural residues (e.g. corn stover and wheat straw), herbaceous energy crops (e.g. switchgrass and miscanthus), short-rotation forestry crops (e.g. hybrid poplar and willow), and the large cellulose/structural components of municipal solid waste. Dedicated energy crops are particularly important due to their potential for large scale availability and low cost. Switchgrass (Panicum virgatum), an abundant perennial warm season grass common across North America, has been considered as particularly attractive for fuels production, with its merits including tolerance to drought and harsh environments, the ability to sequester carbon and conserve soil, and relatively high yields when grown on marginal land (Lynd et al., 2009).

Several challenges impede development of biological conversion of lignocellulosic biomass to ethanol and other valuable products. First, the natural recalcitrance of lignocellulosic biomass has to be overcome by pretreatment, an important unit operation that makes cellulose accessible to cellulase enzymes (Himmel et al., 2007). Although distinctly different in such aspects as reactor configuration, temperature, chemicals applied, reaction times, pH, and effects on biomass, pretreatments by ammonia fiber expansion (AFEX), dilute sulfuric acid, lime, liquid hot water (LHW), soaking in aqueous ammonia (SAA), and sulfur dioxide (SO₂) impregnation all provided very similar yields and thus costs when applied to...
corn stover (Wyman et al., 2005). However, performance was much more variable when the same technologies were employed with poplar wood, with a higher lignin variety proving particularly challenging for most of the pretreatments (Mosier et al., 2005; Wyman et al., 2009).

Although high yields could be realized following biomass pretreatment, the enzyme protein doses applied were, approximately, a quarter pound per gallon (~0.03 kg protein/l or 0.3 kg of enzyme solution per liter based on that the solution containing about 10% protein) of resulting ethanol, too high an amount to be commercially attractive unless enzyme costs become very low (Wyman et al., 2009). Thus, to improve the cost effectiveness of enzymes, pretreatment conditions and subsequent enzymatic hydrolysis must be optimized for high sugar release with much less enzyme. Enzymatic digestion of cell wall carbohydrates with high yields requires the synergy of several categories of enzymes including cellulases, hemicellulases, and accessory hemicellulose debranching enzymes, for example, α-L-arabinofuranosidase, acetyl xylan esterase, feruloyl esterase, and p-coumaroyl esterase (Selig et al., 2008a). The abilities of cellulase to adsorb on cellulose (accessibility) (Chen and Grethlein, 1988; Jeoh et al., 2007) and to hydrolyze cellulose after adsorption (effectiveness) can be considered as the main factors affecting enzymatic saccharification of cellulosic biomass (Kumar and Wyman, 2009a,b). However, both enzyme adsorption onto solids and further their effectiveness for hydrolysis once in place will be strongly affected by substrate and enzyme characteristics and physical parameters, with the result that the digestibility of pretreated solids is strongly influenced by pretreatment efficacy and its effect on substrate features (Kumar et al., 2009).

The results reported here were developed to support the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI), a partnership among leading pretreatment experts from Auburn, Michigan State, Purdue, and Texas A&M Universities; the University of California at Riverside; and the National Renewable Energy Laboratory using switchgrass provided by Ceres, Inc., and enzymes furnished by Genencor, a Danisco Division. The Office of the Biomass Program of the US Department of Energy funded this project to develop comparative data on enzymatic hydrolysis of switchgrass solids following pretreatment by leading technologies. Although various preparations of cellulase and hemicellulase have been tested for cellulose and/or hemicellulose hydrolysis, they were not tailored individually for specific cellulosic feedstocks and pretreatment technologies. In addition, although previous studies showed that performance can vary considerably among pretreatment technologies, enzyme formulations and pretreatments could be better optimized through enhancing our understanding of the interaction between the two. Thus, the objectives of this research were to (1) develop comparative sugar release data on pure cellulose and xylan by cellulases and hemicellulase enzymes used in the CAFI project to better understand enzyme specificity, (2) develop comparative sugar release data for application of these enzymes to switchgrass solids resulting from leading pretreatments, (3) measure cellulase adsorption on switchgrass processed by leading pretreatments, and (4) evaluate the relationship of the digestibility of switchgrass solids from leading pretreatments to removal of lignin, xylan, and acetyl groups and cellulase adsorption.

2. Methods

2.1. Feedstocks

Microcrystalline cellulose (Avicel PH-101, Lot # 1344705) and birchwood xylan (Lot # 038K0751) were purchased from Sigma Chemicals (St. Louis, MO). Phosphoric acid swollen cellulose (PASC) was prepared from Avicel PH101 by soaking in 85 wt.% phosphoric acid followed by precipitation with a large quantity of DI water (Wood, 1988). All chemicals other than those mentioned specifically were of ACS purity and purchased from Sigma Chemicals (St. Louis, MO). Xylooligomers were the room temperature supernatant resulting from birchwood xylan hydrolysis with just water at 190 °C for 15 min (Qing et al., 2010).

Ceres, Inc. (Thousand oaks, CA) provided the Dacotah switchgrass (P. virgatum), a thin stem upland variety, used for the work reported here. The grass was originally planted in December 1999 in Pierre, SD and harvested in May 2008 after standing over the winter. Small square bales were stored in a building, dried at 50 °C to less than 10% moisture, and milled using a knife or hammer mill to pass through a ¼ inch screen. Upon receipt, switchgrass was stored in sealed bags at −20 °C until use. The switchgrass was further milled to pass through a 40 mesh screen and was washed three times to remove free sugars prior to pretreatment by mixing with a mass of hot DI water (80–90 °C) equal to 10 times the wet weight of biomass.

Our CAFI partners generously provided solids resulting from pretreatment of Dacotah switchgrass by the following technologies: ammonia fiber expansion (AFEX) by Michigan State University, dilute sulfuric acid (DA) and sulfur dioxide (SO₂) impregnation by the University of California at Riverside, liquid hot water (LHW) by Purdue University, lime by Texas A&M University, and soaking in aqueous ammonia (SAA) by Auburn University. All pretreated switchgrass solids were washed by mixing with a mass of DI water equal to 10 times the weight of the pretreated solids and filtering off the water; this approach was repeated three times. The pretreatments and corresponding reaction conditions are summarized in Table 1.

2.2. Enzymes

Commercial preparations of Spezyme® CP cellulases (Lot No. 301-05330-206, protein content 82 mg/ml, ~62 FPU/ml), Multifect xylanase (Lot No. 301-04296-205, protein content 27 mg/ml, 25203 OSX/ml), and Accellerase 1000 cellulase (Batch No. 1600877126, protein content 61 mg/ml, 50 FPU/ml) along with a non-commercial preparation of β-xylosidase (Lot No. 20050881-0882, protein content 75 mg/ml), were generously provided by Genencor, a Danisco Division (Palo Alto, CA) for all hydrolysis experiments. Novozyme 188 beta-glucosidase (Batch No. 097K0682, protein content 67 mg/ml, ~665 CBU/ml) was purchased from Sigma–Aldrich. The protein contents of these enzymes were determined from nitrogen-analysis of trichloroacetic acid (TCA) precipitated protein by Genencor and Michigan State University and used to calculate enzyme protein loadings.

2.3. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were run in duplicate at standard conditions (50 °C, 0.05 M citrate buffer, pH 4.8) spooled out in NREL IAP 9 “Enzymatic Saccharification of Lignocellulosic Biomass” (Selig et al., 2008b). Citrate buffer, sodium azide [antimicrobial], enzymes, and DI water were mixed with pretreated solids to produce a final solids loading of about 2% by mass (equivalent to 1 wt.% glucan concentration). A mixture of Spezyme CP and Novozyme 188 enzymes at a FPU:CBU ratio of 1:2 was added to a loading of 30 mg total protein/g glucan in the pretreated biomass. Sugar concentrations were measured in aliquots taken during enzymatic hydrolysis by an Agilent HPLC (1200 Series LC System, Agilent Technologies Inc., Palo Alto, CA) equipped with an Aminex HPX-87H column (Catalog No. 125-0140, 300 × 7.8 mm) and guard cartridge (Catalog No. 125-0129) both purchased from Bio-Rad.
Table 1

Pretreatment conditions, corresponding solids compositions, and component removals following pretreatment of Dacotah switchgrass by leading technologies.

<table>
<thead>
<tr>
<th>Pretreatment technology</th>
<th>Liquid/solid ratio</th>
<th>Temperature (°C)</th>
<th>Chemical loading</th>
<th>Reaction time (min)</th>
<th>Component removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Solids recovery after pretreatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glucan</td>
</tr>
<tr>
<td>None</td>
<td>9</td>
<td>140</td>
<td>1.0 wt.% H2SO4</td>
<td>40</td>
<td>100.0</td>
</tr>
<tr>
<td>DA</td>
<td>9</td>
<td>180</td>
<td>0.05 g SO2 per g biomass</td>
<td>10</td>
<td>60.0</td>
</tr>
<tr>
<td>SO2</td>
<td></td>
<td></td>
<td>NH3</td>
<td></td>
<td>100.0</td>
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<tr>
<td>AFEX</td>
<td></td>
<td></td>
<td>NH4OH</td>
<td></td>
<td>100.0</td>
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<tr>
<td>LHW</td>
<td></td>
<td></td>
<td>O2</td>
<td></td>
<td>100.0</td>
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<td>SAA</td>
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<td>100.0</td>
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<tr>
<td>Lime</td>
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<td>100.0</td>
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</tbody>
</table>

* % Component in solids is based on the remaining solids after pretreatment, except for solids recovery after pretreatment, which is based on starting biomass.

** AIS-acid insoluble lignin.

*** Others include proteins, ash, and uronic acids etc.

Labs (Richmond, CA, USA). Glucan-to-glucose and xylan-to-xylose hydrolysis yields were calculated as defined in Eqs. (1) and (2), respectively, in which the values of 1.111 and 1.136 account for the mass gain during hydrolysis of glucan to glucose and xylan to xylose:

\[
\text{% glucan-to-glucose hydrolysis} = \frac{\text{GH}}{\text{GP} \times 1.111} \times 100 \tag{1}
\]

in which: \( \text{GH} = \text{mass of glucose released in enzymatic hydrolyzate, g.} \)

\[
\text{% xylan-to-xylose hydrolysis} = \frac{\text{XH}}{\text{XP} \times 1.136} \times 100 \tag{2}
\]

in which: \( \text{XH} = \text{mass of xylose released in enzymatic hydrolyzate, g.} \)

2.5. Xylose and xylooligomer inhibition of cellulose hydrolysis

To evaluate xylose and xylooligomer inhibition of enzymatic hydrolysis of cellulose by cellulase, xylose or xylooligomers were mixed with Avicel in a 0.05 M citrate buffer to reach final concentrations of 0–15 g/L and 0–50 g xylose equivalent/L, respectively, consistent with an approach previously applied by Qing et al. (2010). Spezyme CP was loaded at an enzyme loading of 28 mg/g cellulose, and the glucose yield in the first hour was calculated based on Eq. (1). In addition, the effects of xylose or xylooligomer addition on cellulase adsorption were tested by following procedures described in Section 2.4 at a fixed Spezyme CP cellulase loading of 28 mg/g cellulose. A linear model was used.

** Adsorption isotherm models

Cellulase adsorption experiments were conducted in 1.5 ml Eppendorf LoBind microcentrifuge tubes (Catalog # 2866491, Sigma–Aldrich) with a protein loss of less than 3%. An appropriate amount of Spezyme CP cellulase was mixed with Avicel or pretreated solids in 50 mM pH 4.8 citrate buffer to achieve a final enzyme loading of 0–12 mg enzyme protein per ml of final solution (0–1200 mg protein/g glucan in pretreated solids). Adsorption mixtures were incubated at 4 °C for 4 h to allow equilibration, and then solids and liquids were separated by centrifugation. The solid portions were dried in a convection oven at 105 °C overnight, and the protein adsorbed on the pretreated solids was estimated directly using a CHNOS analyzer (Flash EATM 112 N/Protein plus CHNS/O Analyzer, CE Elantech, Lakewood, NJ) with a nitrogen factor (NF) of 7.12 estimated for Spezyme CP following a previously published method (Kumar and Wyman, 2009a).

The Langmuir isotherm equation (Beldman et al., 1987) was used to describe adsorption, with the parameters estimated by the non-linear optimization toolbox in MatLab 7.5 (The MathWorks, Inc., Natick, MA):

\[
E_{\text{bound}} = \frac{\sigma \cdot E_{\text{free}}}{k_d + E_{\text{free}}} \tag{3}
\]

In Eq. (3), \( E_{\text{bound}}, E_{\text{free}}, \sigma, \) and \( k_d \) represent the amount of cellulase adsorbed on the solids (mg/g solids), the cellulase remaining in solution (mg/ml), the maximum cellulase adsorption capacity (mg/g solids), and the equilibrium constant (mg/ml), respectively.
to correlate cellulase adsorption with the concentration of added xylose or xyloooligomers.

### 3. Results and discussion

#### 3.1. Sugar release by hydrolysis of Avicel, PASC, and xylan with cellulases and hemicellulases

Commercially available cellulases and hemicellulases are produced by fungi and are mixtures of an array of cellulose and hemicellulose hydrolytic enzymes (Dien et al., 2008). For example, Spezyme CP is primarily defined as a cellulase because of its high titer of cellulase activities but also has some β-glucosidase and xylanase activities as well as traces of α-arabinofuranosidase, β-xylosidase, and α-galactosidase activities (Dien et al., 2008). As widely known, hydrolysis of crystalline cellulose to glucose with high yields requires the synergistic action of endo- and exo-acting enzymes. In addition, β-glucosidase hydrolyzes cellulobiose and higher soluble cellooligomers to glucose, thus reducing end-product inhibition of the endo- and exo-activities (Baker et al., 1995). If the enzyme mixture is weak in β-glucosidase, external supplementation is important for effective cellulose hydrolysis (Wyman et al., 1986).

Consistent with these profiles, cellulases and hemicellulases gave distinctive sugar release patterns when applied to Avicel, PASC, and xylan. Due to their very low cellulase activities, Multifect xylanase (MX) and β-xylosidase (bX) hydrolyzed very little (<5% after 24 h) Avicel to glucose, as shown in Fig. 1a and b. As expected, Novozyme188 (N188) released negligible amounts of glucose as a result of its lack of endo- and exo-glucanase activities. Although both Spezyme CP (SC) and Accellerase 1000 (A1000) hydrolyzed 12.7 and 13.3% of Avicel, respectively, at 1 h, A1000 outperformed SC in converting cellulobiose into glucose, consistent with its higher β-glucosidase activity (Ko et al., 2009). However, at 24 h, A1000 did only slightly better than SC in terms of total sugar yield.

Fig. 1c and d shows that phosphoric acid swollen cellulose (PASC), which is highly amorphous, was much more easily digested than microcrystalline cellulose, i.e. Avicel, with the result that 39.7% and 45.5% of the possible sugar was released at hour 1 at an enzyme loading of 10 mg/g cellulose with SC and A1000, respectively. However, A1000 produced significantly higher glucose yields than SC due to its higher beta-glucosidase activity (Ko et al., 2009). Interestingly, MX and bX were both able to release 5 to 10% (after 1 h) of the glucose from PASC, much more than from Avicel, most likely due to trace cellulase activities in both commercial hemicellulase preparations (Dien et al., 2008). Similarly, at 24 h, SC hydrolyzed over 50% of the PASC into soluble sugars, while A1000 achieved nearly complete conversion to glucose. It is interesting to note that large amounts of glucose oligomers, mostly cellolbiose, accumulated in the first hour of PASC hydrolysis even for Accellerase. When hydrolyzing amorphous cellulose such as PASC, the extra cellulase activity of Accellerase 1000 reduced cellobiose accumulation and improved performance compared to Spezyme CP.

Breakdown of pure xylan in water has been found to vary considerably with the type of enzyme and to be greatly affected by temperature and solvent acidity (Hespell and Cotta, 1995). As shown in Fig. 2, some interesting trends were found in the first hour after adding different enzymes to xylan. First, about 36% of the pure xylan was released as xyloooligomers after 1 h in pH 4.8 citrate buffer at 50 °C without addition of any enzymes. However, compared to the no-enzyme control, MX addition doubled the amount solubilized to 73.1% of the original xylan with 45.5% of the total being xyloooligomers and the remaining 27.6% being xylose. Interestingly, bX released less xylan sugar (49.0%) than MX and only 13% more xylan was solubilized than for the no-enzyme control. However, as much as 30.4% of the total sugar released was xylose as a result of the superior β-xylosidase activity of bX.
SC and A1000 only increased xylan release by 10–15% compared to the no-enzyme control, with less than 5% being xylose monomers. N188 did not affect overall xylan solublization compared to the no-enzyme control, although it did convert some oligomers to monomeric xylose.

If we examine xylan conversion at 24 h in Fig. 2, we see that MX addition released about 87.1% of the total xylan, 66.3% of which was xylose and the remaining 20.8% was xylooligomers. SC and A100 addition resulted in solubilization of about 69.7% and 73.6% of the total xylan, respectively, but SC produced a higher proportion of xylooligomers than A1000. N188 also showed the ability to hydrolyze slightly more xylan than released from the no-enzyme control and to convert some oligomers to monomeric xylose.

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As demonstrated in this study, significant amounts of xylooligomers accumulated during xylan hydrolysis with individual cellulases and hemicellulases. Although Multifect xylanase seemed to hydrolyze the greatest amount of xylan, significant amounts of xylooligomers remained in solution, especially at the initial stage. Beta-xylosidase was effective in hydrolyzing xylobiose and xylooligomers to xylose, and addition of beta-xylosidase to xylanase seemed to be the key to improve enzymatic hydrolysis of xylan as shown elsewhere (Kumar and Wyman, 2009c). However, none of the enzymes tested completely hydrolyzed xylan, and supplementing cellulase with beta-xylosidase and xylanase has been shown to enhance performance of enzymatic hydrolysis of solids from sulfur dioxide and particularly AFEX pretreatments (Kumar and Wyman, 2009c).

3.2. Effect of xylooligomers on cellulase adsorption to Avicel

A recent study showed that xylobiose and higher xylooligomers strongly inhibit enzymatic hydrolysis of pure cellulose, pure xylan, and pretreated corn stover (Kumar and Wyman, 2009c) and xylooligomers inhibited cellulose hydrolysis much more than xylose (Qing et al., 2010; Ximenes et al., 2010). However, the inhibition mechanism of xylooligomers on enzymatic hydrolysis of cellulose is not yet clear. Xylooligomers have been shown to have a strong impact on cellulase activity and possibly xylanase activity as well (Suh and Choi, 1996). Recent research in our laboratory shows that competitive inhibition appears to be responsible for the slowdown in glucose release, especially at the initial stage. Furthermore, the structural similarity of xylan and xylooligomers to cellulose or cellobiose could tie up cellulase and β-glucosidase and thus impact cellulase effectiveness (Qing et al., 2010).

Fig. 3a records a drop in cellulase adsorption when xylooligomers and xylose were added to cellulose hydrolysis. In this case,
addition of 15 g/L of xylooligomers lowered cellulase adsorption from 18.7 to 3.8 mg cellulase/g cellulose, while xylose had a very small impact over the range of concentrations applied. As shown in Fig. 3b, a linear correlation ($R^2 = 0.97$) was observed between hydrolysis yields and cellulase adsorption for addition of xylose and xylooligomers during the first hour. The results from this work suggest that strong inhibition of cellulose hydrolysis by xylooligomers could be partially attributed to decreasing cellulase adsorption. However, further investigation is needed to clarify the inhibition kinetics.

3.3. Compositions of solids from pretreatment of switchgrass by CAFI technologies

Table 1 shows the different pretreatments resulted in distinct differences in lignin, glucan, xylan, arabinan, and acetyl contents in the pretreated solids. Pretreated solids under acidic conditions for dilute acid (DA), sulfur dioxide (SO$_2$), and liquid hot water (LHW) had very low xylan contents of 2.5–4.5% but high glucan contents of 50.1–53.9%. For pretreatments with alkaline chemicals, i.e. soaking in aqueous ammonia (SAA) and lime, the solids remaining after pretreatment had lignin contents of only about 14%, xylan levels close to that in the feedstock of about 22%, and enriched glucan contents of 53.0–55.6%. Ammonia fiber expansion (AFEX) pretreatment of switchgrass resulted in virtually no compositional change in switchgrass except for negligible xylan loss and minor acetyl removal. However, as shown in previous studies, improvements in digestibility by AFEX pretreatment are possibly due to relocation of lignin, decrystallization of cellulose, and a phase change in the crystal structure from cellulose I to cellulose III as a result of cellulose swelling (Dale et al., 1996; Chundawat, 2009).

Table 1 also summarizes the effect of the different pretreatments studied here on the removal of major biomass components. First, we can see that DA, SO$_2$, LHW, lime, and SAA solubilized about 35–40% of the switchgrass. Pretreatments at acidic conditions, i.e. DA, SO$_2$, and LHW, led to nearly complete (>90%) xylan and arabinan removal but only removed about 13.0–18.6% of the original lignin. However, SAA and lime pretreatments at alkaline conditions removed 55.1–59.3% of the original lignin plus 38.0–39.9% of the original xylan. In addition, alkali pretreatments removed carboxylic acid substitutions, e.g. acetyl groups and uronic acids, from hemicellulose in addition to some of the hemicellulose, with improved enzyme access to hemicellulose and cellulose (Kim and Holtzapple, 2006; Kumar et al., 2009). In summary, low pH pretreatments removed a major portion of hemicellulose, and high pH pretreatments except AFEX removed a large part of the lignin plus some xylan. However, the extent of these effects varied with substrate and pretreatment, in line with many previous studies (Kumar et al., 2009; Mosier et al., 2005; Wyman et al., 2005).

3.4. Enzymatic hydrolysis of switchgrass solids pretreated by various technologies

As shown in Fig. 4, the digestibility of CAFI3 pretreated switchgrass varied considerably with pretreatment technologies, with 1 h glucose yields from enzymatic digestion spanning a wide range of from 16.3–33.5% at a Spezyme CP plus Novozyme 188 loading of 30 mg of protein/g glucan in the pretreated solids. The lowest glucose yield was 16.3% for AFEX treated solids, while lime pretreatment gave the highest glucose yield of 33.5% followed in order of decreasing yields by SO$_2$, SAA, DA, and LHW. After 24 h, the lowest digestibility was 47.4% for AFEX pretreated solids, while SO$_2$ gave the highest yields of 95% followed, in order, by lime, LHW, DA, and SAA.

Fig. 5 plots the first hour glucose release for enzymatic hydrolysis against compositional changes, i.e. xylan, lignin, or acetyl group removal, for the solids resulting from pretreatment of switchgrass by CAFI3 technologies. No consistent trends were seen relating first hour hydrolysis yields to xylan removal, and the data appear to be quite scattered (Fig. 5a). A somewhat more apparent trend can be seen of increased 1 h glucose release with increased lignin removal, particularly for AFEX, DA, LHW, and lime (Fig. 5b). However, SO$_2$ demonstrated a greater rate of digestion than this trend would suggest while SAA did not fare well even though it removed more lignin. The relationship between first hour glucose release and acetyl group removal was quite strong for AFEX, SAA, SO$_2$, and lime, but DA and LHW had considerably lower digestion than expected from this trend line (Fig. 5c).

Enhanced glucan digestibility with xylan removal is often reported (Allen et al., 2001; Yang and Wyman, 2004), with improving cellulose accessibility by enzymes having been historically noted (Jeoh et al., 2007) while recent attention has been drawn to the possibility of reducing the strong inhibition of cellulose by xyloligomers (Kumar and Wyman, 2009c). However, when examined across different pretreatments in this study, no clear correlation was seen between xylan removal and digestibility, likely due to the effects of the pretreatments tested on other substrate properties in addition to xylan.

Many believe that lignin is one of the key contributors to biomass recalcitrance and one of the primary substrate features impacting enzymatic conversion of cellulosic biomass (Himmel et al., 2007). Lignin impacts not only cellulose accessibility, due to its protective sheathing and hydrophobic nature, but also cellulase effectiveness as a result of unproductive binding (Chang and Holtzapple, 2000; Kumar et al., 2009; Mansfield et al., 1999). Furthermore, lignin removal and associated removal of cross linkages with carbohydrates has often been shown to improve cellulose digestibility, with mechanisms postulated to be both improved cellulose accessibility and enhanced cellulase effectiveness (Chang and Holtzapple, 2000; Kumar et al., 2009; Ohgren et al., 2007; Yang and Wyman, 2004).

Removal of acetyl content has been reported to enhance digestibility (Wood and McCrae, 1986), and recent reports of its positive impact on cellulose accessibility indicate that acetyl removal enhanced cellulose accessibility and cellulase effectiveness for corn stover hydrolysis with purified CBH (Kumar and Wyman, 2009a). The benefit of acetyl removal may be through reducing inhibition of xylanase and debranching enzymes (Mitchell et al.,
In addition, Selig and coworkers in a recent study reported enhanced hydrolysis due to synergism between acetyl xylan esterase and cellobiohydrolase (CBHI) (Selig et al., 2008a). Furthermore, Kumar and coworkers showed that acetyl removal increased cellulose accessibility and also increased xylanase effectiveness, resulting in increased xylan removal that in turn enhanced glucan accessibility (Kumar et al., 2009). This study suggested a positive correlation between acetyl removal and switchgrass digestibility that was common to all of the tested pretreatments, with the exception of weaker relationships for DA and LHW, in general agreement with many of the previous investigations (Chang and Holtzapple, 2000; Kumar et al., 2009; Wood and McCrae, 1986). However, it should be noted that the removal of acetyl groups during these pretreatments is not exclusive and is often accompanied by changes in other substrate features.

3.5. Adsorption of cellulase on CAFI3 pretreated switchgrass and relation to digestibility

Cellulase adsorption on cellulose (accessibility) (Chen and Grethlein, 1988; Jeoh et al., 2007) and its hydrolysis ability after it is adsorbed (effectiveness) (Kumar and Wyman, 2009b) are considered to be the primary factors affecting enzymatic saccharification of cellulosic biomass. However, enzyme adsorption onto solids and further their effectiveness can be affected by substrate and enzyme features and physical parameters, as reviewed elsewhere (Kumar et al., 2009). Although several previous papers described enhancement of cellulose accessibility to enzymes, many found...
that cellulase adsorption varied substantially with the substrate and pretreatment type.

In this study, cellulase adsorption is plotted against cellulase loadings of 0–12 mg/ml (0–1200 mg/g glucan in pretreated solids) in Fig. 6a for pretreated switchgrass solids. From this data, adsorption parameters based on the Langmuir model, i.e. the maximum cellulase adsorption capacity \( \sigma \) and equilibrium constant \( k_d \) were calculated, as listed in Table 2. We see that the values varied substantially with pretreatment type. For instance, AFEX pretreated switchgrass had the lowest maximum cellulase adsorption capacity of 95.6 mg/g solids, while the value was highest for SO2 pretreatment of Dacotah switchgrass solids. From this data, cellulase adsorption varied substantially with the substrate and pretreatment type.

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\[ \text{Table 2} \]

<table>
<thead>
<tr>
<th>Pretreatment technology</th>
<th>Maximum cellulase adsorption capacity, ( \sigma ) (mg/g substrate)</th>
<th>Equilibrium constants, ( k_d ) (mg/ml)</th>
<th>( k_{h1} ) (mg/ml)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicel</td>
<td>85.8</td>
<td>0.9</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>153.7</td>
<td>3.3</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>SO2</td>
<td>170.9</td>
<td>2.3</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>AFEX</td>
<td>95.6</td>
<td>0.9</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>LHW</td>
<td>138.0</td>
<td>1.6</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>SAA</td>
<td>157.4</td>
<td>2.1</td>
<td>0.90</td>
<td></td>
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<tr>
<td>Lime</td>
<td>135.4</td>
<td>5.0</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

4. Conclusions

Application of cellulases and hemicellulases to Avicel, PASC, and xylan resulted in distinctive sugar release patterns. Significant amounts of xylooligomers accumulated during xylan hydrolysis, and the strong inhibition of cellulose hydrolysis by xylooligomers could be partially attributed to xylanomers decreasing cellulase adsorption. The digestibility of CAFl3 pretreated switchgrass varied considerably with pretreatment technologies but could not be consistently related to xylan, lignin, or acetyl group removal. Initial hydrolysis rates did correlate with cellulase adsorption capacities for all pretreatments except lime. Further investigation is needed to relate this behavior to physical and compositional properties of pretreated switchgrass.

Acknowledgements

References


