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Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars

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ABSTRACT

For this project, six chemical pretreatments were compared for the Consortium for Applied Fundamentals and Innovation (CAFI): ammonia fiber expansion (AFEX), dilute sulfuric acid (DA), lime, liquid hot water (LHW), soaking in aqueous ammonia (SAA), and sulfur dioxide (SO_2). For each pretreatment, a material balance was analyzed around the pretreatment, optional post-washing step, and enzymatic hydrolysis of Dacotah switchgrass.

All pretreatments + enzymatic hydrolysis solubilized over two-thirds of the available glucan and xylan. Lime, post-washed LHW, and SO_2 achieved >83% total glucose yields. Lime, post-washed AFEX, and DA achieved >83% total xylose yields. Alkaline pretreatments, except AFEX, solubilized the most lignin and a portion of the xylan as xylo-oligomers. As pretreatment pH decreased, total solubilized xylan and released monomeric xylose increased. Low temperature-long time or high temperature-short time pretreatments are necessary for high glucose release from late-harvest Dacotah switchgrass but high temperatures may cause xylose degradation.

1. Introduction

The world supply of fossil fuels is limited and will eventually fail to meet the global demand for energy, which continues to increase each year (Asif and Muneer, 2007). Because of this, it is necessary to research and develop alternative fuel sources before supplies become severely constrained. Bioethanol is one of the possible renewable alternatives to liquid fossil fuels. The majority of current world-wide production is derived from starch-based (e.g. corn) or sugar-based materials (e.g. sugar cane), but it is also possible to use lignocellulosic materials, such as agricultural and forestry residues, grasses, and trees as feedstocks. For the biological conversion route, a three-step process is necessary to adequately convert the cell wall sugars in these materials to ethanol: pretreatment followed by enzymatic hydrolysis and fermentation. Pretreatment is a process, either biological, chemical, physical, thermal, or some combination of these, which disrupts the cell wall structure and increases enzyme access to the cell wall carbohydrates, the substrate for lignocellulosic ethanol (Alvira et al., 2010; da Costa Sousa et al., 2009; Mosier et al., 2005; Yang and

Abbreviations: 5-HMF, 5-hydroxymethylfurfural; AFEX, ammonia fiber expansion pretreatment; CAFI, Consortium for Applied Innovation and Fundamentals; CBU, cellobiase unit; DA, dilute sulfuric acid pretreatment; DP, degree of polymerization; DBP, dry biomass entering pretreatment; FPU, filter paper unit; Glc, glucose; GO, gluco-oligomers; LHW, liquid hot water pretreatment; SAA, soaking in aqueous ammonia pretreatment; SO_2, sulfur dioxide pretreatment; XO, xylo-oligomers; Xyl, xylose.

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Wyman, 2008). The choice of pretreatment can have a significant impact on biorefinery costs (Aden and Foust, 2009; Eggeman and Elander, 2005) and most other processing decisions including feedstock selection, choice of enzymes and microbes, and waste treatment applications (Yang and Wyman, 2008). Because of the cost and pervasive impact of pretreatment on all aspects of the process, the choice of pretreatment method is extremely important. But this decision is hardly straightforward as there are a large number of pretreatment options currently available, each of which has certain advantages and disadvantages and some of which lend themselves better to certain feedstocks (Alvira et al., 2010; da Costa Sousa et al., 2009; Yang and Wyman, 2008).

In order to effectively compare different pretreatment methods, it is important to conduct an accurate material balance, tracking the fate of cellulose and hemicellulose throughout the process and generating accurate yields. Many pretreatment methods result in liquid streams and the amount and type of components that are solubilized are dependent on the method. So if process yields are calculated based on the initial biomass composition without taking into account any pretreatment mass losses which are due to solid–liquid separation or post-washing, the results will be erroneous. One mass balance method is the carbon balance which tracks all of the carbon-based compounds in all of the process streams (Hatzis et al., 1996). Closing this balance can be difficult because of the complexity of measuring all of the carbon-based compounds. One study reports significant error-related issues when closing this balance for cellulase production (Schell et al., 2002). Another method that has been employed by previous CAFI projects and other pretreatment researchers is to measure the individual components, focusing on only those of interest such as glucose/glucan, xylose/xylan, other sugars and lignin (Zhang et al., 2009; Zhu et al., 2010). Another option is to calculate a total biomass mass closure based on the solids content of each stream. But this is a less reliable method because of the difficulty involved in quantifying the solids content of dilute liquid or solution streams.

It is often difficult to compare pretreatment methods based on literature because of the inconsistency in materials and methods used. The intent of the Consortium for Applied Fundamentals in Innovation (CAFI) projects was to provide a consistent basis for comparing a number of different thermochemical pretreatment methods (Wyman et al., 2005b). For these comparisons, each pretreatment method was carried out on the same feedstock, used the same enzymes and microbes during enzymatic hydrolysis and fermentation, and employed the same, consistent protocols wherever applicable throughout the process. The feedstocks used for the previous two CAFI projects were corn stover – an agricultural residue (Wyman et al., 2005a), and poplar – a hardwood (Wyman et al., 2009). This third round of the CAFI project examined and compared the effect of pretreatment and enzymatic hydrolysis of different varieties of switchgrass. There have been a number of recent papers that have looked at the feasibility of processing switchgrass for bioethanol (Bals et al., 2010; Himmelsbach et al., 2009; Xu et al., 2010; Yang et al., 2009) and also a recent review that discusses some of the older papers (Keshwani and Cheng, 2009).

The goal of the portion of the CAFI III project reported in this manuscript was to conduct material balances around six thermochemical pretreatment methods: ammonia fiber expansion (AFEX) at Michigan State University, dilute sulfuric acid (DA) and sulfur dioxide (SO_2) at University of California-Riverside, lime at Texas A&M University, liquid hot water (LHW) at Purdue University, and soaking in aqueous ammonia (SAA) at Auburn University. The objective was to compare the process yields and stream characteristics for the combined stages of pretreatment and enzymatic hydrolysis of switchgrass, using the same feedstock (Dacotah switchgrass), enzymes, and analytical methods. Because washing following pretreatment may not be necessary to improve digestibility for all pretreatment methods with all feedstocks, post-washing was analyzed separately from pretreatment.

2. Methods

2.1. Dacotah switchgrass

Dacotah switchgrass, an upland variety, was planted in 1999 in Pierre, SD by Ceres, Inc. (Thousand Oaks, CA) The material used for these experiments was produced in 2007 and harvested in late winter of 2008 after standing on the field over winter. Composition analysis of the switchgrass was performed by Ceres according to the standard NREL protocols (Sluiter et al., 2010). The samples provided by Ceres had initially been milled to pass through a 0.25 in. (6.35 mm) screen and were shipped to each participating university. At each university, prior to performing the pre-wash, the switchgrass was milled to pass through a 2 mm screen using either a knife mill or coffee grinder. This secondary size reduction was performed so the biomass would be the appropriate particle size for composition analysis throughout the process.

2.2. Pre-wash

A pre-wash step was performed at each university to remove any soluble sugars which could mask the solubilization of cell wall sugars. Batches of Dacotah switchgrass (200 g each) were soaked in 2 L of 80–90 °C distilled water for 10–15 min. The switchgrass slurry was vacuum-filtered through Whatman No. 1 filter paper (Whatman Ltd.). This process was repeated three times and after each wash step, a portion of the filtrate was retained for oligomeric sugar analysis. The washed solids were dried in a 45 °C oven. The extracted weight loss of the switchgrass was determined by subtracting the dry weight of the washed switchgrass and the dry mass loss to the filter paper from the initial dry weight.

2.3. Solids composition

The composition of the pre-washed switchgrass (structural sugars and lignin) was determined using the standard two-stage extraction followed by a two-step acid hydrolysis (Sluiter et al., 2010). The composition of the solids following the pretreatment, post-wash, and enzymatic hydrolysis was determined using the same method, but the extraction step was not performed due to the potential loss of soluble biomass components.

2.4. Soluble total and oligomeric sugar analysis

Oligomeric sugar analysis was conducted using either the standard NREL method for oligomeric sugar determination of liquid streams (Sluiter et al., 2008) which uses an autoclave based acid hydrolysis, or a modified version of this method. The modified method was identical except that it was scaled down to use 2 mL of sample and assays were run in duplicate in 10 mL screw-cap culture tubes. The tubes were incubated in a 121 °C bench-top hot plate for one hour, cooled on ice, and filtered into HPLC vials. The oligomeric sugar concentration was determined by subtracting the monomeric sugar concentration of the non-hydrolyzed samples from the total sugar concentration of the acid hydrolyzed samples.

2.5. HPLC analysis

The monomeric and total (monomeric + oligomeric) sugar concentrations were determined for the pre-wash liquid, pretreatment liquor, post-wash liquid, and enzymatic hydrolysate. HPLC samples were analyzed using a Bio-Rad (Hercules, CA, USA) Aminex

HPX-87H column equipped with appropriate guard columns. Degassed 5 mM H_2SO_4 was used as the mobile phase and the column temperature was held at 60 °C. Glucose, xylose (plus galactose and mannose), and arabinose concentrations were determined for each liquid stream. Because the xylose, galactose, and mannose peaks cannot be separated using the HPX-87H column (Irick et al., 1988), any results reported for xylose also includes mannose and galactose. For grasses the galactose and mannose contents tend to be very low – in sum less than 1.5% of the total biomass (Biomass, 2006).

2.6. Pretreatment and post-washing

The pretreatment and post-wash conditions for all six pretreatment methods are listed in Table 1. The basis for the mass balance was 100 kg dry biomass entering pretreatment (DBP) in stream A. Water use and catalyst loading for pretreatment are also reported on this basis. The experimental details on each pretreatment method are reported in Supplementary material (Annex 1). Except for AFEX and SAA where pretreatment conditions were chosen to limit hemicellulose degradation, pretreatment conditions were chosen to optimize sugar yields based on preliminary experiments with Dacotah switchgrass which are not detailed here.

For pretreatments which produced a slurry, a solid–liquid separation was performed following pretreatment (except for SAA where washing is integrated with the pretreatment). For the whole slurry without post-washing, the entire slurry was weighed following pretreatment. While keeping the sample well-mixed, a sample was removed for enzymatic hydrolysis. The weight and moisture content of the remaining slurry was determined. The slurry was vacuum-filtered through Whatman No. 41 or No. 4 filter paper. The volume of the filtrate was determined and samples were taken for monomeric and oligomeric sugar analysis. The retained solids were washed with 500 mL of distilled water (20–30 °C) per 10 g of dry solids. The volume of the filtrate was determined and samples were taken for monomeric and oligomeric sugar analysis. The values for monomeric and oligomeric sugars solubilized by the pretreatment were calculated as the sum of the sugars from the initial slurry filtrate and the washed solids filtrate. The solids were dried overnight at 50 °C. The total wet weight and the %-solids content of the retained solids and filter paper were determined.

Essentially the same method used for unwashed samples was employed for washed, pretreated solids undergoing enzymatic hydrolysis, but the methods differed in three details. Samples were not removed for enzymatic hydrolysis prior to filtration, the retained solids were washed with the wash water after the first filtration step as specified by the pretreatment method, and the washed solids were not dried prior to enzymatic hydrolysis.

2.7. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted in triplicate in 250 mL Erlenmeyer flasks. The pretreated biomass solids or slurry was loaded at 1% glucan loading followed by enough distilled water to bring the total volume to roughly 80% of the final volume (~120 mL), including the water already present in the biomass/slurry. The pH was adjusted to between 4.5 and 5.0 using 50 mM sodium citrate buffer after which antibiotics were added to each flask (600 μL of 10 g/L tetracycline and 450 μL of 10 g/L cycloheximide). Distilled water was added to each flask to bring the final volume (after addition of the enzymes) to 150 mL. The flasks were tightly sealed with rubber stoppers and secured with tape before being placed in a shaking incubator which was set at 200 rpm and 50 °C. After the temperature in the flasks reached 50 °C, the flasks were removed and the enzymes were added based on the glucan content in the pre-washed, untreated biomass. Spezyme® CP (Batch: 301-05330-206; Genencor Division of Danisco US, Inc, NY, USA), with a protein content of 82 mg/mL and specific activity of 50 FPU/mL was loaded at 15 FPU g^{-1} glucan in untreated biomass. β-Glucosidase (Novozyme® 188, Novozymes Corp.) with a protein content of 67 mg/mL and specific activity of 600 CBU/mL was loaded at 30 CBU g^{-1} glucan in untreated biomass. The protein content of the enzymes was determined from total N analysis using the Dumas method for combustion of nitrogen to NO_x following trichloroacetic acid (TCA) precipitation to remove non-protein nitrogen. Because the enzymes were added based on the glucan content in the untreated biomass, the enzyme loading based on the glucan in the pretreated biomass was variable for each feedstock. Table 2 shows the protein loading in terms of the both glucan content and the (glucan + xylan) content of each pretreated feedstock. After adding the enzymes, the flasks were re-sealed and placed in the incubator for 168 h.

Following the incubation period, the flasks were removed from the incubator and the flask contents were transferred to disposable centrifuge tubes. The samples were centrifuged at 10,000 rpm for

Table 2
Enzyme loadings for pretreated solids on the basis of the polymeric sugars in the pretreated and washed biomass (where applicable). Enzyme loadings for all samples were on the basis of 15 FPU Spezyme CP/g glucan in dry biomass entering pretreatment (DBP) and 30 CBU Novozyme 188/g glucan in DBP.

	Enzyme loading (mg protein/g polymeric sugar in pretreated [washed] biomass)			
	Glucan		Glucan + Xylan	
	Spezyme CP	Novo 188	Spezyme CP	Novo 188
Washed				
AFEX	24.1	3.2	16.4	2.2
DA	24.4	3.5	22.4	3.2
Lime	21.0	3.4	15.5	2.5
LHW	29.8	3.1	28.4	2.9
SAA	21.2	3.4	15.2	2.4
SO_2	23.3	3.3	21.7	3.1
Unwashed				
AFEX	22.9	3.0	14.4	1.9
LHW	29.6	3.0	27.8	2.8

Table 1
Pretreatment and post-wash comparison.

Method	Pretreatment						Post-wash	
	Temp (°C)	Time (min)	Catalyst	Water/solids loading	Catalyst loading[a]	Water use[b]	Post-wash water use[b]	Water temp (°C)
AFEX	150	30	Anhydrous NH_3	2 g H_2O/g DBP	152	200	1174	100
DA	140	40	1% H_2SO_4	10% solids (w/w)	9	891	3000	20–25
Lime	120	240	$Ca(OH)_2$	15 g H_2O/g DBP	100	1468	4655	20–25
			O_2		100 psi			
LHW	200	10	Water	15% solids (w/w)	N/A	663	3069	80–90
SAA	160	60	15% Aqueous NH_3	10% solids (w/v)	135	765	10,000	20–25
SO_2	180	10	5% SO_2	10% solids (w/w)	5	895	3000	20–25

[a] Catalyst loading: kg/100 kg DBP.
[b] Water use: L/100 kg DBP.

30 min. Afterward, the volume and weight of the supernatant were recorded and samples were taken for monomeric (aliquot 1) and oligomeric sugar analysis (aliquot 2). The flasks were washed with 25 mL of distilled water to remove any residual solids. The wash liquid was transferred into the original centrifuge tubes containing the hydrolysis solids and the mixture was re-suspended, following which the samples were centrifuged a second time. The weight and volume of the supernatant was determined and samples were taken for monomeric (aliquot 3) and oligomeric (aliquot 4) sugar analysis. Aliquots 1 and 3 were heated at 100 °C for 15 min to denature the enzymes and cooled in a freezer for 15 min. After cooling, the samples were transferred into HPLC shell vials and stored at −20 °C until HPLC analysis. Acid hydrolysis was performed on aliquots 2 and 4 for oligomeric sugar determination. The values of the monomeric and (monomeric + oligomeric) sugars were calculated as the sum from aliquots 1 + 3 and aliquots 2 + 4, respectively.

The centrifuge tubes with the solids were placed in the freezer overnight. The next day, the solids were removed from the tubes and allowed to thaw. The total weight and moisture content of the solids was recorded. The solids were dried at 50 °C overnight following which acid hydrolysis was performed to determine the solids composition.

2.8. Mass balance calculations

For the mass balance, an inventory of key system components, including water, was compiled for all streams when possible. The actual data generated for each pretreatment is reported as the average of any replicates and included in Supplementary material (Tables S1–S8). Because of space limitations standard deviations are not included in the tables. Previous CAFI publications (Wyman et al., 2005a, 2009) have divided the mass balance into two stages, and for the purpose of comparison, process yields for this paper have been reported in the same manner. Stage 1 consists of the combined pretreatment and post-washing and Stage 2 is enzymatic hydrolysis (Fig. 1). No data are reported for streams 3, 7 and C when no post-wash step was performed. Gluco- (GO) and xylo-oligomers (XO) were reported in monomeric equivalents.

Process sugar yields for each stage were calculated based on the pre-washed, dry biomass entering pretreatment (DBP) in stream A using the following equations with subscripts to indicate the stream. The equations are simplified by stating the ratio of the molecular weights of glucose to glucan (180/162) as (1/0.9) and xylose to xylan (150/132) as (1/0.88). Because sucrose that is present in the biomass can also contribute glucose to the liquid streams, this amount was determined by multiplying the amount of sucrose in the biomass by (180.2/342.3), the ratio of the molecular weights of glucose and sucrose.

$$\text{Stage 1 Glucose Yield } (\%) = \frac{\text{Glucose}_6 + \text{Glucose}_7 + \text{GO}_6 + \text{GO}_7}{(\text{Glucan}_A/0.9) + \text{Glucose}_A + \text{Sucrose}_A * (180.2/342.3)} * 100\% \quad (1)$$

$$\text{Stage 1 Xylose Yield } (\%) = \frac{\text{Xylose}_6 + \text{Xylose}_7 + \text{XO}_6 + \text{XO}_7}{(\text{Xylan}_A/0.88)} * 100\% \quad (2)$$

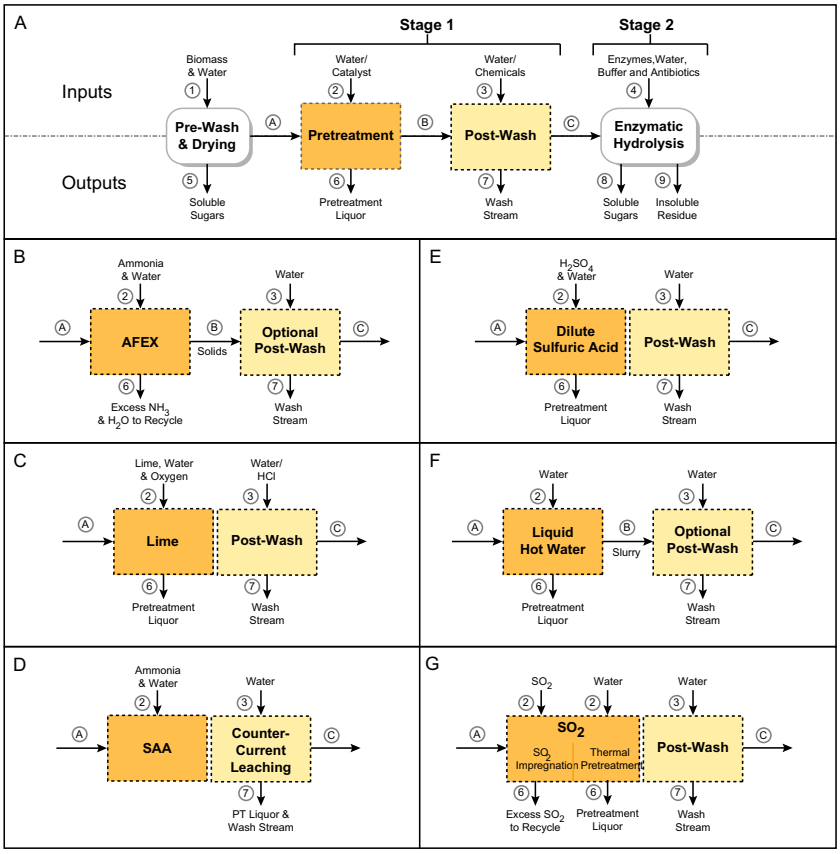

Fig. 1. Pretreatment input–output diagrams. (A) General pretreatment diagram. Inputs and outputs to the process are indicated by numbered streams while lettered streams indicate streams internal to the process. The pre-wash step and enzymatic hydrolysis step were common to all pretreatments. (B–F) Specific pretreatment diagrams for AFEX, DA, lime, LHW, SAA and SO_2. Only the streams which were reported in the mass balance tables are included in the input–output diagrams (with the exception of the excess NH_3 and H_2O stream for AFEX (6) and the excess SO_2 stream in SO_2 pretreatment (6) which were not reported due to difficulties in measuring gas stream data). AFEX and LHW pretreatments reported two sets of data, one set with a post-wash step and one set without.

$$\text{Stage 2 Glucose Yield } (\%) = \frac{\text{Glucose}_8 + \text{GO}_8}{(\text{Glucan}_A/0.9) + \text{Glucose}_A + \text{Sucrose}_A * (180.2/342.3)} * 100\% \quad (3)$$

$$\text{Stage 2 Xylose Yield } (\%) = \frac{\text{Xylose}_8 + \text{XO}_8}{(\text{Xylan}_A/0.88)} * 100\% \quad (4)$$

The overall mass closure for glucose and xylose was based on the process from pretreatment through enzymatic hydrolysis (pre-washing was not included). Mass closure was calculated as the soluble sugars (monomers and oligomers as monomeric equivalents) in the liquid streams (6, 7, and 8) plus the glucan or xylan in the hydrolysis solids (stream 9 – converted to monomeric equivalents), divided by the amount of polymeric sugars (as monomeric equivalents) and soluble sugar in the dry biomass in stream A.

Lignin composition is difficult to determine for liquid streams, therefore the amount of removed lignin was calculated as the difference between the lignin in the solid residue entering and leaving the stage (either Stage 1 or Stage 2), divided by the lignin in stream A.

3. Results and discussion

3.1. Pre-wash and stage 1 – pretreatment and post-wash

A pre-wash step may be a desirable step for feedstocks which have a high soluble sugar content that could mask the solubilization of cell wall sugars. However, Dacotah switchgrass is a mature grass sample, and the initial soluble sugar content was quite low (Table 3) so the amount of glucose detected in the hydrolyzed wash stream ranged from only 0.4 to 1.1 kg glucose per 100 kg DBP. For similarly mature samples, a pre-wash step may not be necessary; however, even a small amount of soluble glucose or sucrose should be included in the material balance. The range in glucan content of the pre-washed switchgrass which was reported by each university was 2.7 kg/100 DBP and the range in xylan content was 3.9 kg/100 DBP. The large range in results is likely indicative of slight differences in feedstock, equipment, and processes between universities.

Pretreatment and post-wash conditions were highly variable between the different methods (Table 1). The pretreatments can be grouped based on their temperature–time combinations: high-temp/short-time (LHW and SO_2); moderate-temp/moderate-time (AFEX, DA, and SAA); and low-temp/long-time (lime). Water use by the pretreatments was lowest for AFEX, highest for lime, with the other pretreatments within a similar range. Catalyst use also varied, with no use by LHW which relies on hydrothermal breakdown of the cell wall structure, a small amount used by DA and SO_2, and the greatest amounts used by the alkaline pretreatments (AFEX, lime, and SAA). Because of this high use, catalyst recycle is considered a necessary part of these processes and would add additional capital cost to the alkaline pretreatment systems (Eggeman and Elander, 2005).

Solids recovery following pretreatment with or without post-washing was around 60% for most of the methods (Table 4), except for lime and washed AFEX which were 10% and 20% higher, respectively, and unwashed AFEX which retained all of the biomass with a small increase due to ammonia incorporation. The composition of the LHW pretreated biomass was not strongly impacted by washing, which is not surprising given the similarity in the solids recovery between the washed and unwashed biomass. However, for AFEX pretreatment the relative proportion of glucan and lignin increased as hemicellulose was removed. The composition of the washed and pretreated solids from the acidic pretreatments (DA, SO_2 and LHW) had higher glucan and lignin contents due to greater solubilization of xylan during Stage 1 (Fig. 2B) and the alkaline pretreatments (lime, SAA) had a higher glucan content due to hemicellulose and lignin removal (Fig. 2C).

Table 3
Composition of unwashed and pre-washed Dacotah switchgrass (% of total DM).

Component	Unwashed	Pre-washed Dacotah Switchgrass					
		AFEX	DA	Lime	LHW	SAA	SO_2
Glucan	35.0	37.1	36.5	37.2	35.6	34.8	36.5
Xylan	21.8	25.5	22.7	23.7	22.6	22.1	22.7
Arabinan	3.5	3.0	3.2	2.5	3.1	3.4	3.2
Acid-Insoluble Lignin	21.4	23.4	20.7	20.8	22.8	21.1	20.7
Sucrose	1.5	–	–	–	–	–	–
Other[a]	15.6	11.0	16.9	15.7	15.9	18.6	16.9

[a] "Other" for the unwashed biomass includes extractives, ash and acetyl, as determined and provided by Ceres. "Other" for the pre-washed biomass was calculated as the difference between the sum of the listed values and 100%.

Table 4
Solids yields and composition following pretreatment and post-washing (where applicable) for the different pretreatment technologies.

Component	Untreated[a]	Post-Washed						Unwashed	
		AFEX	DA	Lime	LHW	SAA	SO2	AFEX	LHW
Solids recovery (kg/100 kg DBP)	–	83.3	60.4	74.4	59.0	64.2	60.5	101.3[b]	61.3[c]
Pretreated solids composition (%)									
Glucan	36.3	45.4	52.3	48.4	50.0	53.7	54.5	39.2	48.5
Xylan	23.2	21.1	4.5	17.3	2.5	21.2	4.1	23.3	3.1
Arabinan	3.1	3.1	0.0	2.0	0.0	2.3	0.0	2.8	0.0
Lignin	21.6	23.3	29.5	12.8	31.5	13.4	26.8	20.2	32.3

[a] The value for the untreated biomass is provided for the purpose of comparison and was calculated as the average of the composition values provided by all of the universities for the pre-washed biomass (Table 3).
[b] The increase in the solids content for AFEX pretreated biomass is due to the incorporation of nitrogen into the biomass during the pretreatment via reactions with ammonia.
[c] The solids recovery for the unwashed LHW whole slurry represents the solids fraction of the pretreatment slurry, all of which goes into enzymatic hydrolysis.

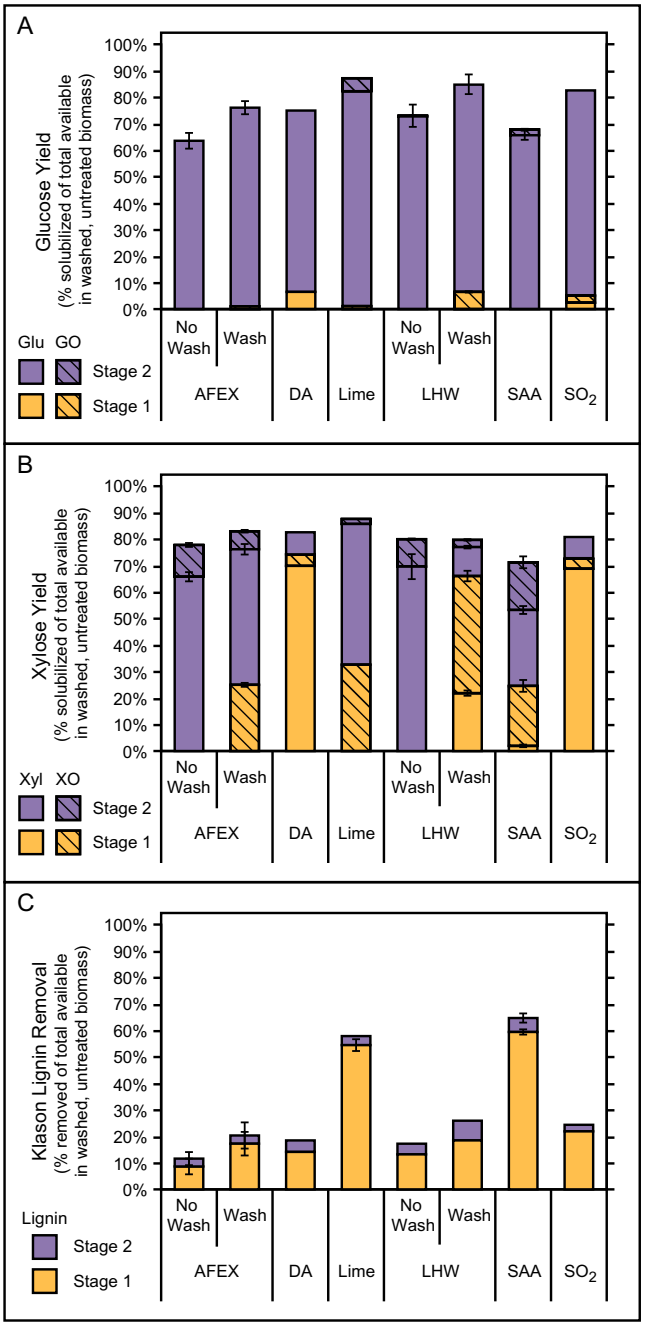

Fig. 2. Glucose and xylose yields and Klason lignin removal from Dacotah switchgrass for Stage 1, Stage 2 and the overall process. (A) Glucose Yield, (B) Xylose Yield, (C) Klason Lignin Removal. Yields include all solubilized monomers and oligomers (in monomeric equivalents) and are expressed as a percentage of the sugar present in the pre-washed, untreated dry biomass. Standard deviations were not reported for DA or SO_2, lime (except for Stage 1 lignin), or LHW (lignin Stages 1 and 2). The Stage 1 lignin values for the unwashed LHW and AFEX material is the lignin that has become more readily removed during the acid hydrolysis quantification method. This material, while made more soluble during Stage 1, is actually removed into the enzymatic hydrolysate during Stage 2. Glu, glucose; GO, gluco-oligomers; Xyl, xylose; XO, xylo-oligomers.

Very little glucan was solubilized during any of the pretreatments (Table 5). The only appreciable amounts solubilized were by DA, SO_2, and LHW pretreatments, but in all cases this was $\leqslant$7% of the total glucose in the untreated biomass. Following pretreatment and post-washing, more than two-thirds of the xylan was removed from biomass pretreated via DA, LHW, and SO_2, while less than one-third was released from AFEX, lime, and SAA (Fig. 2B). As the pH decreased from a strongly acidic pH (DA and SO_2), to a pH closer to neutral (LHW), to an alkaline pH (AFEX, lime, and SAA), the total amount of solubilized xylan decreased and the proportion of the solubilized sugars that were in oligomeric form increased (Table 6). It is known that as pretreatment severity increases (due to increased temperature, time and/or decreased pH) and more xylan is removed from the biomass, the solubilized xylo-oligomers are simultaneously deconstructed from higher to lower degrees of polymerization (DP), eventually resulting in monomeric xylose and degradation products (Kabel et al., 2007). Lignin solubilization follows the opposite trend, as a greater amount was removed at an alkaline pH (Fig. 2C). This pattern of lignin and hemicellulose solubilization with respect to pretreatment pH has been reported elsewhere (Pedersen and Meyer, 2010; Wyman et al., 2005a, 2009). This pattern is represented by a simple model in Fig. 3. For simplicity, this model does not show changes which may occur to cellulose with respect to pH.

3.2. Stage 2 – enzymatic hydrolysis

For all pretreatments, the majority of the glucose was solubilized during Stage 2 – enzymatic hydrolysis (Table 5 and Fig. 2A). Of the pretreatments, only the lime and SAA enzymatic hydrolysates contained any measurable amounts of gluco-oligomers. This amount was fairly low and may indicate either some inadequacy in the enzymes used or some error with respect to the oligomeric sugar quantification. The hydrolysate from unwashed and washed AFEX, unwashed LHW, and SAA all contained large amounts of oligomeric xylose (Table 6 and Fig. 2B). This indicates that at the enzyme loadings used, Spezyme CP and Novo188 were not able to adequately break down all of the hemicellulose, which is not unexpected as these enzymes have been shown to possess low hemicellulase activity and are particularly slow at degrading xylo-oligomers (Qing and Wyman, 2011). The addition of hemicellulases to the enzyme mixture has been shown to increase glucose yields from pretreated materials that have higher hemicellulose content, such as those produced by AFEX and ammonia recycle percolation (ARP), the precursor to SAA (Kumar and Wyman, 2009b). In another study on AFEX pretreated switchgrass, supplementation with hemicellulases was necessary to achieve the highest sugar yields during enzymatic hydrolysis (Bals et al., 2010). A more optimal enzyme mixture could increase the release of sugars from these materials.

Post-washing of AFEX-treated and LHW-treated biomass, which solubilized roughly 20–30% of the lignin as well as large quantities of xylo-oligomers (~30–50% of the total xylose), had a marked effect on glucose yields, increasing them by around 10–15%. This increase could be due to a number of reasons including increasing enzyme accessibility to the cell wall structure and removal of enzyme inhibitors such as lignin-based compounds, sugar degradation products, xylose, and xylo-oligomers. However, the reason for the increase in yields is most likely not due to the removal of low molecular weight lignin-based inhibitors. At low solids loadings such as those used here, these compounds have not been shown to strongly inhibit enzymes during enzymatic hydrolysis (Hodge et al., 2008). Another possibility is that the hot water washing removed inhibitory xylo-oligomers (Kumar and Wyman, 2009a; Qing et al., 2010), and additionally improved the accessibility of the biomass to the enzymes by removing additional biomass components.

None of the pretreatment methods was able to solubilize over 90% of the available glucose or xylose into oligomeric and monomeric form. However, it is apparent that either the combination of low temperature/long residence time or high temperature/short residence time is important for releasing glucose from the substrate. The glucose yields were highest (>80%) for the

Table 5
Glucose solubilization at each process stage expressed as the amount of glucose released in terms of the amount present in pre-washed, untreated dry biomass (kg/100 kg DBP).

	Stage 1					Stage 2			Overall solubilization			Glucose mass closure (%)
	Pretreatment		Post-wash		Stage 1 total	Enzymatic hydrolysis		Stage 2 total				
	Glc[a]	GO[b]	Glc[a]	GO[b]		Glc[a]	GO[b]		Glc[a]	GO[b]	Total	
AFEX												
No Wash[c]	N/A	N/A	N/A	N/A	N/A	26.4	0.0	26.4	26.4	0.0	26.4	100
Wash[d]	N/A	N/A	0.0	0.5	0.5	30.9	0.0	30.9	30.9	0.5	31.4	108
DA												
Wash[d]	2.0	0.2	0.5	0.1	2.8	27.7	0.0	27.7	30.2	0.3	30.5	92
Lime												
Wash[d]	0.1	0.1	0.0	0.4	0.6	33.4	2.0	35.4	33.5	2.5	36.0	108
LHW												
No wash[c]	N/A	N/A	N/A	N/A	N/A	28.8	0.1	28.9	28.8	0.1	28.9	99
Wash[d]	0.2	2.5	0.0	0.0	2.7	31.0	0.0	31.0	31.2	2.5	33.7	95
SAA[e]												
Wash[d]	–	–	0.0	0.1	0.1	25.3	0.8	26.1	25.3	0.9	26.2	94
SO_2												
Wash[d]	0.8	0.8	0.2	0.2	2.0	31.7	0.0	31.7	32.7	1.0	33.7	91

[a] Glc, solubilized glucose.
[b] GO, solubilized gluco-oligomers, reported in monomeric equivalents.
[c] No wash, no post-wash step following pretreatment.
[d] Wash, post-wash step following pretreatment.
[e] SAA pretreatment liquor was not sampled – the post-wash value is combined pretreatment liquor and post-wash liquid.

Table 6
Xylose solubilization at each process stage expressed as the amount of xylose released in terms of the amount present in pre-washed, untreated dry biomass (kg/100 kg DBP).

	Stage 1					Stage 2			Overall solubilization			Xylose mass closure (%)
	Pretreatment		Post-Wash		Stage 1 total	Enzymatic hydrolysis		Stage 2 total				
	Xyl[a]	XO[b]	Xyl[a]	XO[b]		Xyl[a]	XO[b]		Xyl[a]	XO[b]	Total	
AFEX												
No Wash[c]	N/A	N/A	N/A	N/A	N/A	19.1	3.5	22.6	19.1	3.5	22.6	95
Wash[d]	N/A	N/A	0.0	7.3	7.3	14.8	2.0	16.8	14.8	9.3	24.1	98
DA												
Wash[d]	14.6	0.9	3.5	0.2	19.2	2.2	0.0	2.2	20.3	1.1	21.4	86
Lime												
Wash[d]	0.0	7.2	0.0	1.7	8.9	14.2	0.5	14.7	14.2	9.4	23.6	97
LHW												
No Wash[c]	N/A	N/A	N/A	N/A	N/A	17.9	2.7	20.6	17.9	2.7	20.6	87
Wash[d]	3.0	7.0	2.7	4.3	17.0	2.8	0.7	3.5	8.5	12.0	20.5	81
SAA[e]												
Wash[d]	-	-	0.6	5.7	6.2	7.2	4.5	11.7	7.7	10.2	17.9	80
SO_2												
Wash[d]	14.6	0.8	3.2	0.2	18.8	2.1	0.0	2.1	19.9	1.0	20.9	84

[a] Xyl, solubilized xylose.
[b] XO, solubilized xylo-oligomers, reported in monomeric equivalents.
[c] No wash, no post-wash step following pretreatment.
[d] Wash, POST-wash step following pretreatment.
[e] SAA pretreatment liquor was not sampled – the post-wash value is combined pretreatment liquor and post-wash liquid.

pretreatments which operated at either of these temperature/time combinations (lime, washed LHW and SO_2). The lowest yields (<70%) were for unwashed AFEX and SAA. Xylose yields were highest (>83%) from lime, washed AFEX, and DA, although all of the pretreatments solubilized more than 80% of the total xylose. While these values may seem low, it is important to keep in mind that the Dacotah switchgrass used for these experiments was harvested in the spring after over-wintering on the field, which can have a strong negative impact on the digestibility of herbaceous biomass (Le Ngoc Huyen et al., 2010).

The glucose mass closure values for all pretreatments were 100 ± 9%. The extreme values were for DA and SO_2 (low), and washed AFEX and lime (high), and may be due to compounded errors within the method, particularly with respect to acid hydrolysis used for composition data. It is possible that yields may be underestimated for the samples with low mass closure and overestimated for the samples with high mass closure. The xylose mass closure was between 80% and 98%, slightly lower than calculated for glucose. This may be due to high temperature degradation of xylose and xylo-oligomers into other compounds. While it is less of an issue for alkaline pretreatments, degradation of xylose (Kabel et al., 2007; Lloyd and Wyman, 2005) and production of inhibitory furans, such as furfural from pentoses and 5-hydroxymethyl furfural (5-HMF) from hexoses, can be significant for pretreatments such as DA, SO_2, and LHW that operate at low pH and high temperatures (Chen et al., 2007; Du et al., 2010; Kabel et al., 2007). For the 5 min residence time LHW pretreatment without post-washing, the furfural concentration in the pretreatment liquor was

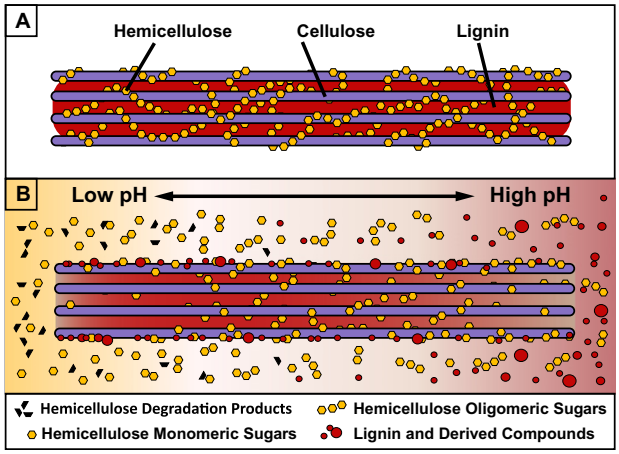

Fig. 3. Cell wall model showing the general effect of pH on solubilization of hemicellulose and lignin. (A) Untreated cell wall and (B) cell wall during pretreatment. Cellulose can also be degraded under extremely acidic conditions; however that is not portrayed in this diagram. Designed based on figures from Mosier et al. (2005) and Pedersen and Meyer (2010).

0.23 g/L. For the 10 min residence time pretreatment, the concentration increased to 2.75 g/L – an amount equal to the degradation of 7.4% of the xylose initially present in the biomass. When this amount is included in the mass balance as an output, the xylose mass closure for LHW pretreatment increased to 95%. Compared to furfural, the amount of HMF produced after the 10 min residence time was very low (0.23 g/L) and accounted for only 0.4% of the glucose initially present in the untreated biomass. For this project, quantification of furfural and HMF for all of the pretreatments was unintentionally overlooked; however, in future work on pretreatment mass balances, particularly those at low pH and high temperatures, at least these two compounds should be included due to their potential impacts on the glucose and xylose mass closure.

Apart from the differences in sugar yields, the differences in solubility profiles for the different pretreatments could have a large impact on the process economics. One issue with respect to pretreatment wash streams is whether it is economically worthwhile or possible to recover the hemicellulose sugars for use later in the process. Pretreatments which solubilize the hemicellulose sugars as oligomers and wish to recover them for fermentation will either require a subsequent step to convert them to a monomeric form or use a micro-organism which can utilize oligomeric sugars. However for those pretreatments that retain the hemicellulose, there are also challenges associated with co-fermentation of a mixture of glucose and xylose (Jin et al., 2010). The solubilization of lignin is also important as the lignin solids are generally modeled as the source of biorefinery electricity and energy for steam production (Aden and Foust, 2009; Eggeman and Elander, 2005). Greater solubilization of lignin could lead to a decreased energy production and increased wastewater treatment costs. However, when conducting simultaneous saccharification and fermentation, retention of the lignin with the biomass can have a negative effect on the fermentation microbes (Jin et al., 2010). When comparing pretreatments, the overall sugar yields are just one factor that should be considered and in the end, there are many tradeoffs with respect to the different methods.

4. Conclusions

All pretreatments solubilized > 2/3 of the available glucan and xylan. Lime, washed LHW, and SO_2 achieved > 83% total glucose yields. Lime, washed AFEX, and DA achieved > 83% total xylose yields. Washing improved glucose release from LHW and AFEX pretreated switchgrass. Pretreatment pH effects the solubilization of biomass components. As pH decreases, solubilized lignin decreases, while total solubilized xylan and released monomeric xylose increases. Differences in pretreatment solubilization impact other processing areas and the process economics. Low temperature-long time or high temperature-short time pretreatment is necessary for high glucose release from late-harvest Dacotah switchgrass, but high temperatures may cause xylose degradation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.04.002.

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