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Sugar yields from dilute sulfuric acid and sulfur dioxide pretreatments and subsequent enzymatic hydrolysis of switchgrass

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ABSTRACT

Dacotah switchgrass was pretreated with sulfuric acid concentrations of 0.5, 1.0, and 2.0 wt.% at 140, 160, and 180 °C and with 1 and 3 wt.% sulfur dioxide at 180 °C over a range of times. Sulfur dioxide loadings of 0%, 1%, 3%, 5%, and 10% wt.% of dry biomass were also tested at 180 °C for 10 min. Sugar yields were tracked for pretreatment and subsequent enzymatic hydrolysis to identify conditions for the highest total sugar yields. Pretreatment with 1 wt.% dilute sulfuric acid at 140 °C for 40 min followed by enzymatic hydrolysis with 48.6 mg enzyme/g initial glucan in raw biomass resulted in ~86% of theoretical yield for glucose and xylose combined. For sulfur dioxide pretreatment, the highest total sugar yield of about 87% occurred at 5% SO_2 for 10 min and 180 °C. However, xylose yields were higher at shorter times and glucose yields at longer times.

1. Introduction

Cellulosic biomass is a unique renewable resource suitable for large-scale sustainable production of low cost liquid fuels compatible with our existing transportation infrastructure, and cellulosic biomass derived fuels (e.g., cellulosic ethanol) offer powerful potential benefits including enhanced energy security, reduced trade deficits, enhanced global competitiveness, rural employment, and reduced greenhouse gas emissions (Lynd et al., 2009). A key to economical and profitable production of biofuels and other products in a bio-refinery is the availability of large amounts of cellulosic biomass (Wyman, 2007). Perennial C4 grasses, such as switchgrass and Miscanthus, offer superior yields because C4 metabolism enables significantly higher yields than the more common C3 metabolism found in most plants, especially under water-limiting conditions (Anderson and Akin, 2008). Switchgrass (*Panicum virgatum*), an abundant perennial adapted to different geographical regions across North America, is considered as an attractive feedstock for bio-refineries because of its tolerance to drought, its ability to improve soil, and its relatively high yields on marginal land (Laser et al., 2009). Switchgrass is also favored because life cycle studies demonstrated that it can enhance carbon sequestration on marginal lands, thus considerably increasing GHG benefits for the first 20 years after crop establishment (Wu et al., 2006).

Pretreatment by disrupting the naturally resistant structure of lignocellulosic biomass to make its cellulose and hemicellulose susceptible to enzymatic hydrolysis and generate fermentable sugars is critical to high yields from biological processes (Yang and Wyman, 2008). With respect to biological ethanol production from lignocellulosic biomass by hydrolysis and fermentation, a good pretreatment must assure high yields of hemicellulose and cellulose sugars from the coupled operations of pretreatment and enzymatic hydrolysis with the lowest possible enzyme doses applied in the latter (Wyman, 2007). Numerous pretreatments have been applied previously to switchgrass including, lime, sodium hydroxide, dilute acid, hydrothermolysis, ammonia recycled percolation, soaking in aqueous ammonia, ammonia fiber expansion, ammonia–hydrogen peroxide percolation, and ionic liquids (Keshwani and Cheng, 2009; Singh et al., 2009). However, only a few of these have been optimized for combined glucose and xylose recovery from both stages, i.e., pretreatment and enzymatic hydrolysis (Hu et al., 2008; Jensen et al., 2010). Furthermore, to our knowledge, material balance closure on the sugar streams for dilute acid pretreatment of switchgrass has not been reported in the literature until the present.

Sulfur dioxide pretreatment has been proven to significantly improve sugar yields compared to non-catalyzed water only pretreatment. In addition, impregnation with gaseous sulfur dioxide can be more effective than with H_2SO_4 in terms of rapid, uniform distribution in cellulosic biomass and offers better recyclability,

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although recovery and recycle costs can be high. The effects of sulfur dioxide during pretreatment include removal of a major portion of hemicellulose sugars, disruption of the lignin–carbohydrate complex, and substantial depolymerization and sulfonation of lignin (Zhu et al., 2009). Sulfur dioxide pretreatment, has been applied to pretreat an array of cellulosic biomass and shown good adaptability to various feedstocks, including agricultural residues such as corn stover (Bura et al., 2002; Ohgren et al., 2005) and sugarcane bagasse (Martin et al., 2002); hardwood (De Bari et al., 2007) and softwoods (Boussaid et al., 1999; Clark et al., 1989; Soderstrom et al., 2002; Stenberg et al., 1998) with representative results summarized in Table 1. In a recent study, the effect of biomass moisture was tested on the performance of SO_2-catalyzed steam explosion of switchgrass, and the results showed that increased moisture content increased permeability of the biomass to SO_2 (Ewanicka and Bura, 2010). In these studies, sulfur dioxide pretreatment was applied over the range of 180–220 °C for 2–10 min at sulfur dioxide loadings of 1–6% of biomass dry weight, with sugar yields as high as 80–90% for almost all feedstocks tested. However, to our knowledge, sulfur dioxide pretreatment conditions have not been optimized for switchgrass, particularly to maximize sugar yields from pretreatment and enzymatic hydrolysis steps combined. In addition, little information is available to compare sulfur dioxide and dilute sulfuric acid pretreatments when applied to identical feedstocks with identical enzyme sources, loadings, and formulations and using the same methods.

In this paper, data are reported on sugar yields from Dacotah switchgrass for sulfuric acid and sulfur dioxide pretreatments, and the results are compared to performance data reported elsewhere for other leading pretreatment technologies that were applied to the same feedstock (Wyman et al., in press). We record recovery of glucose and xylose sugars in both pretreatment and subsequent enzymatic digestion and reinforce that instead of focusing on only xylose in the first stage or glucose in the second, as typically reported, it is vital to consider cumulative yields of these sugars from both stages (Lloyd and Wyman, 2005). We also report the effect of acid loading on performance in terms of total sugar release with the goal of identifying possible paths to reduce its use and seek to optimize enzyme loadings and formulations. The present paper focuses on (1) optimizing dilute acid and sulfur dioxide pretreatments to maximize total glucose plus xylose yields from both pretreatment and enzymatic hydrolysis, (2) finding enzyme loadings that maximize sugar yields at these optimal pretreatment conditions, and (3) closing material balances at optimal conditions to verify the results.

2. Methods

2.1. Feedstock

Dacotah switchgrass (*P. virgatum*), a thin stem upland variety, was provided by Ceres, Inc. (Thousand Oaks, CA). This material was originally planted in December, 1999 in Pierre, SD (44.367966°N 100.336378°W) and harvested in May, 2008 after standing over the winter. Small square bales were stored in a building, dried at 50 °C to around 5% moisture, and milled using a Knife or hammer mill to pass through ¼ inch screen. Upon receipt at the University of California at Riverside, the switchgrass was divided into about 1 kg quantities that were sealed in plastic freezer bags at −20 °C until use. Upon thawing out, the switchgrass was further milled to pass through a 2 mm screen by a Thomas Wiley Laboratory Mill (Model No. 4, Thomas Scientific, Swedesboro, NJ) prior to pretreatment.

2.2. Dilute acid pretreatment

Sulfuric acid concentrations of 0.5, 1.0, and 2.0 wt.% were applied at 140, 160 and 180 °C over a range of five different pretreatment times to give a total of 45 different conditions. The pretreatment times were chosen to span a range of combined pretreatment severities from −0.3–3.0 that past experience suggested would bracket times that would give the highest total glucose plus xylose yields from the combined operations of pretreatment and enzymatic hydrolysis. The logarithm of the combined severity parameter (log CS) was calculated from the pretreatment temperature T (in °C), pretreatment time t (in min), and room temperature pH of the biomass slurry recorded after pretreatment as follows (Chum et al., 1990):

$$\log \mathrm{CS} = \log\left(t \cdot e^{\frac{T-100}{14.75}}\right) - \mathrm{pH} \quad (1)$$

Acid solution was combined with switchgrass (5% solid loading) in tubular reactors made of ½ inch diameter × 6 inch length Hastelloy tubes. The tubes were sealed with Teflon plugs and stainless steel caps and then left overnight at room temperature for dispersion of acid catalyst in the biomass. The sulfuric acid loading in wt.% was based on the amount mixed with water prior to adding biomass and compensated for the moisture in the switchgrass. All pretreatments were run in triplicate in tubular reactors that were heated to reaction temperature using two sand baths, the first set to 260 °C for rapid heat up to the target temperature at which time the tubes were transferred to a second sand bath set ca. 2 °C higher than the pretreatment temperature to hold the reaction at the target temperature as measured by a thermocouple. The heat up time in the first sand bath varied between 1 and 3 min and is not included in the stated reaction times or the severity calculation. After pretreatment, the reactors were quenched by quickly transferring them to a room temperature water bath until the temperature dropped to 30 °C (the cooling time was around 1–2 min and was not included in the stated reaction time). To separate solids from liquid, the slurry was immediately filtered (Whatman GF/F-pore size 0.7 μm, Piscataway, NJ). The undiluted filtrate was collected and stored at 4 °C for further analysis of monomeric and oligomeric sugars. The collected solids were washed with room temperature deionized (DI) water using an amount equal to 10 times the biomass weight before pretreatment. All experiments were run in duplicate or triplicate, with average data reported.

Table 1
Representative results reported in the literature for sulfur dioxide pretreatment of cellulosic biomass.

Feedstock	Pretreatment conditions	Sugar recovery[a]	Reference
Softwood	215 °C, 3 min with 2.55 wt.% SO_2 (*Pinus radiata*);	G: 82%; 57 g total sugars/100 g biomass	Clark et al. (1989)
	195 °C, 4.5 min with 4.5% SO_2 (Douglas Fir);	>85% hemicellulose sugars	Boussaid et al. 1999
Hardwood	210 °C, 2 min with 1.6 wt.% SO_2 (Aspenwood);	G: 37 g/100 g; X: 10.3 g/100 g	De Bari et al. (2007)
	190 °C, 5 min with 3 wt.% SO_2 (CAFI standard poplar)	>90% sugar recovery	Wyman et al. (2009)
Sugarcane bagasse	205 °C, 10 min with 1 wt.% SO_2	52.9 g/100 g	Martin et al. (2002)
Corn stover	190 °C, 5 min with 2% SO_2 (on wet basis);	84% total G and X yield;	Ohgren et al. (2005)
	190 °C, 5 min with 6% SO_2	81% total G and X yield	Bura et al. (2002)

[a] G: glucose, X: xylose, % yields are sugar recovered based on sugar available; g/100 g is yield of sugar recovered from 100 g of dry biomass.

2.3. Sulfur dioxide pretreatment

For sulfur dioxide pretreatment, Dacotah switchgrass was mixed with about five times its weight of room temperature DI water and then pressed to remove extra water using a customized squeezer until a moisture level of about 65 wt.% was reached. This moist processing did not affect the switchgrass composition (data not shown). The moist prewashed Dacotah switchgrass was then impregnated with 0–10 wt.% (or 0–0.1 g SO_2 per gram dry grass) gaseous sulfur dioxide (SO_2, >99% pure, Matheson Tri-Gas, Newark, CA) and held overnight at room temperature in sealed heavy duty Ziploc bags. Prior to pretreatment, impregnated Dacotah switchgrass was carefully transferred to a 1 L Parr reactor made of Hastelloy C (Parr Instruments, Moline, IL) and mixed with DI water to a final solid loading of 10 wt.% on a dry basis. Runs with 1% and 3% wt.% sulfur dioxide pretreatment were carried out at 180 °C over a range of five different pretreatment times (0–60 min) to give a total of 10 different pretreatment conditions. Additionally, sulfur dioxide loadings of 0%, 1%, 3%, 5%, and 10% wt.%, of dry biomass weight (0, 0.01, 0.03, 0.05, and 0.1 g sulfur dioxide per gram dry grass) were tested at 180 °C for just 10 min. The combinations of sulfur dioxide loadings and pretreatment times were chosen to span a range of log CS from −0.7 to 2.3 with the log combined severity, log CS, defined by Eq. (1) as a function of pretreatment temperature T (in °C), pretreatment time t (in min), and pH of the biomass slurry recorded at room temperature after pretreatment.

Sulfur dioxide pretreatments were run at 180 °C, with heat provided by lowering the Parr reactor into a 4-kW fluidized sand bath (model SBL-2D, Techne Co., Princeton, NJ), as described in more detail elsewhere (Yang and Wyman, 2009). The biomass slurries were stirred at 200 rpm with two 40 mm diameter pitched blade impellers stacked on top of each other and rotating to pump the material downwards. The reactor temperature was monitored with a K-type thermocouple. The heat up time was about 2 min, and this time was not included in the stated reaction time. After pretreatment, the reactor was quenched in a room temperature water bath until the temperature dropped to 80 °C (The cooling time was around 2–3 min and was not included in the stated reaction time). The slurry was vacuum filtered immediately through a glass fiber filter (Whatman GF/F-pore size 0.7 μm, Piscataway, NJ) with the temperature being held above 60 °C. The collected solids were washed with room temperature DI water using an amount equal to 10 times the biomass weight before pretreatment.

2.4. Enzymes

A commercial preparation of Spezyme® CP cellulase (Lot No. 301-05330-206, protein content 82 mg/ml, specific activity ~0.76 FPU/mg) was generously provided by Genencor, a Danisco Division (Palo Alto, CA), for all hydrolysis experiments. Novozyme® 188 β-glucosidase (Batch No. 097K0682, protein content 67 mg/ml, specific activity ~9.93 CBU/mg) was purchased from Sigma–Aldrich. Protein contents of these enzymes, measured by Genencor and Dr. Bruce Dale's group at Michigan State University using the nitrogen-analysis of trichloroacetic acid (TCA) precipitated protein, were used to calculate enzyme protein loadings. Specific activities in units/mg of these enzymes were based on previous enzyme activity measurements by our laboratory (Kumar and Wyman, 2009).

2.5. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were run in duplicates by following NREL LAP 9 "Enzymatic Saccharification of Lignocellulosic Biomass" at NREL standard conditions (50 °C, 0.05 M citrate buffer, pH 4.8) (Selig et al., 2008). Citrate buffer (final molarity 50 mM), sodium azide (antimicrobial, final concentration of 0.01 g/L), enzymes, and DI water were mixed with pretreated solids to achieve a final solids loading of around 2% (equivalent to 1% (w/w) glucan concentration). For optimizing pretreatment conditions, high enzyme loadings were applied to pretreated switchgrass solids. For dilute acid pretreated switchgrass, a mixture of Spezyme CP cellulase plus Novozyme 188 β-glucosidase at a FPU:CBU ratio of 1:2 was added at a loading of 68.4 mg protein/g glucan in the raw biomass. For sulfur dioxide pretreated switchgrass, a mixture of Spezyme CP plus Novozyme 188 at a FPU:CBU ratio of 1:2 was applied at 88.0 mg protein/g glucan in the raw biomass. For optimizing enzyme loadings at selected pretreatment conditions and for comparing dilute acid and sulfur dioxide pretreatments, enzymatic hydrolysis was performed over a range from 4.8 to 96.6 mg total protein/g glucan in the raw biomass for Spezyme CP plus Novozyme 188 at a FPU:CBU ratio of 1:2. Sugar concentrations for aliquots taken after 72 h of enzymatic hydrolysis were measured 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 a micro-guard cartridge (Catalog No. 125-0129, 30 × 4.6 mm) in a standard cartridge holder (Catalog No. 125-0131, Bio-Rad Labs, Richmond, CA, USA). After 72 h of hydrolysis, the remaining solids were collected by filtering through a 0.7 μm glass fiber filter (Whatman GF/F-pore size 0.7 μm, Piscataway, NJ) and washed with a large volume of DI water to remove residual sugars. The solids were then dried overnight at 105 °C and analyzed for Klason-lignin, glucan, and xylan compositions.

2.6. Compositional analysis

The percentages of glucan, xylan, and Klason-lignin in raw Dacotah switchgrass, the pretreated solids, and the hydrolysis residues were measured in duplicates according to the standard NREL LAP "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter et al., 2008b). The liquid streams from pretreatment and enzymatic hydrolysis were analyzed for glucose and xylose using an HPLC after neutralization with calcium carbonate. Furthermore, part of the liquid was post-hydrolyzed according to the NREL LAP "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples" to obtain a solution with only monomeric sugars, which was again analyzed by HPLC (Sluiter et al., 2008a). The difference between the monomers following post-hydrolysis and before is the amount of oligomers.

2.7. Sugar yield calculations

For clarity in reporting these results, the terms Stages 1 and 2 refer to pretreatment of the Dacotah switchgrass and enzymatic hydrolysis of the pretreated solids, respectively, in this paper. Glucose and xylose yields were reported for the two Stages separately, i.e., Stage 1 glucose, Stage 1 xylose, total Stage 1 glucose plus xylose, Stage 2 glucose, Stage 2 xylose, total Stage 2 glucose plus xylose, and the total glucose plus xylose from the two stages combined, i.e., combined Stage 1 + 2 glucose plus combined Stage 1 + 2 xylose. Furthermore, yields of glucose and xylose were defined as the total amount of sugar monomers and oligomers together and expressed as monomer equivalents. For instance, Stage 1 xylose yield was the combination of xylose and xylo-oligomers released in the pretreatment hydrolysate and converted to xylose equivalents.

All yields were reported in two ways. In one, sugar mass yields were defined as grams per 100 gram of raw Dacotah switchgrass to provide a perspective on sugar release from that feedstock. Based on the original feedstock composition of 35.0% glucan and 21.8% xylan, the maximum potential mass yield of glucose and xylose

on their monomeric sugar basis was 38.9 and 24.8 g, respectively, per 100 g of the raw Dacotah switchgrass giving a combined total of 63.7 g sugar per 100 g of raw grass. In the second definition, sugar yields were calculated as a percent of the maximum potential total glucose plus xylose from the original raw Dacotah switchgrass to provide a perspective on the relative contribution of each sugar to total sugar production. Thus, for the Dacotah switchgrass used, the maximum possible percent yield of glucose would be 61.1% while the maximum possible percent yield for xylose would be 38.9% of the total possible for these two sugars.

2.8. Material balances

The conditions that gave the highest total Stage 1 plus Stage 2 sugar yields, for both dilute acid and sulfur dioxide pretreatment, were repeated and carefully analyzed to support detailed mass closure. The percentages of glucan, xylan, and Klason-lignin in raw Dacotah switchgrass, the pretreated solids, and the hydrolysis residues were measured according to standard NREL LAP (Sluiter et al., 2008b). All of the data was recast on the basis of 100 g of raw switchgrass to facilitate comparisons and interpretation. For the pretreatment filtrate and enzymatic hydrolyzate liquid streams, glucose and xylose concentrations were quantified and expressed in terms of monomeric sugars, also based on 100 g starting biomass. The overall glucan, xylan, and sugar balance closures were expressed in terms of the percentage of each component that could be accounted for in all the solid and liquid streams from the combined pretreatment and enzymatic hydrolysis operations.

3. Results and discussion

3.1. Sugar yields from dilute acid pretreatment and subsequent enzymatic hydrolysis

Fig. 1a illustrates the Stage 1 sugar yields from Dacotah switchgrass pretreated with 1.0 wt.% dilute sulfuric acid solution for 140, 160, and 180 °C over pretreatment times of 1–60 min. Three sets of conditions gave virtually the same maximum total glucose plus xylose yields from just pretreatment: 140 °C for 40–60 min, 160 °C for 5–10 min, and 180 °C for 2.5 min. At any of these pretreatment conditions, the highest total glucose plus xylose yield from just pretreatment was about 24.0 g per 100 g raw switchgrass, which is about 37.6% of the theoretical maximum total sugar yield. More interesting trends can be seen for the individual glucose or xylose yields. Glucose release during pretreatment was minor for pretreatment at 140 and 160 °C, with only about 3 g of glucose released even after a long pretreatment time of 40–60 min. However, at the higher temperature, glucose release increased substantially, with up to 12.5 g of glucose released from switchgrass at 180 °C for 20 min, amounting to almost one third of the available glucan in the raw switchgrass, probably due to depolymerization of amorphous glucan contained in the switchgrass (Weil et al., 1994). However, some of the glucose may have come from hemicellulose, i.e., xyloglucan. Xylose yields peaked at 20.8 and 20.0 g/100 g dry biomass (>80% of total available xylan) after pretreatment at 180 °C for 2.5 min and at 160 °C for 5 min, respectively, both with 1 wt.% sulfuric acid. After that, xylose yields dropped off rapidly, indicating degradation at the more severe pretreatment conditions, particularly at the highest temperature. Xylose yields peaked at 20 min for pretreatment at 140 °C and stayed flat for longer pretreatment times of 20–60 min suggesting that the xylose generation rate is similar to its degradation rate for a long period of time for pretreatment at 140 °C.

Fig. 1b shows the Stage 2 sugar yields from enzymatic hydrolysis of the solids left following switchgrass pretreatment. Because most of the xylan was hydrolyzed to xylose and dissolved oligomers in Stage 1, xylose mass yields from Stage 2 were low at less than 2.4 g/100 g original total for all pretreatment conditions. For 180 °C pretreatment, negligible amounts of xylose were recovered in Stage 2, due to the very low amount left in the pretreated solids. Glucose was by far the major sugar released in Stage 2, and its yields peaked at short pretreatment times for high temperature pretreatment. For example, a mass yield of as high as 30.7 g glucose/100 g total dry raw switchgrass was achieved for enzymatic hydrolysis of the solids produced by pretreatment at 180 °C for 1–2.5 min. Glucose yields dropped steadily if switchgrass was pretreated for times longer than 10 min, most likely due to significant release of glucose in Stage 1. The highest glucose mass yield was 32.2 g/100 g dry switchgrass following pretreatment for 20 min at 160 °C, while the time to the maximum yield increased to

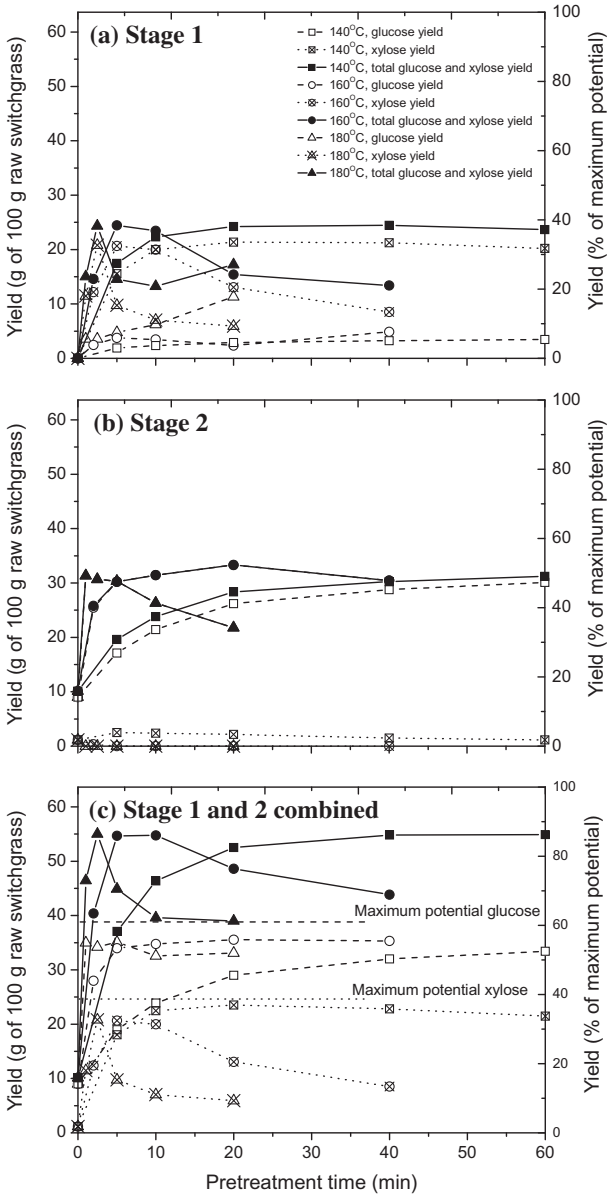

Fig. 1. (a) Stage 1 glucose and xylose yields for pretreatment of Dacotah switchgrass with 1.0% sulfuric acid for different temperature/time series; (b) Stage 2 glucose and xylose yields for enzymatic hydrolysis of the solids from pretreatment of Dacotah pretreated with 1.0% sulfuric acid for different temperatures and times; (c) Total glucose plus xylose yields from Stage 1 and 2 combined for pretreatment of Dacotah switchgrass with 1.0% sulfuric acid at an enzyme loading of 68.4 mg/g glucan in raw switchgrass.

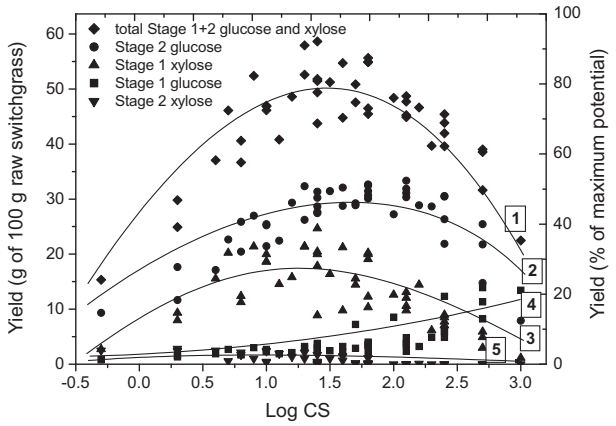

Fig. 2. Glucose plus xylose yields plotted against the log combined severity (log CS) parameter for all temperature/acid time series run for dilute acid pretreatment and subsequent enzymatic hydrolysis of Dacotah switchgrass with lines drawn to reflect approximate data trends. Trendlines 1–5 represent total Stage 1 + 2 glucose plus xylose, Stage 2 glucose, Stage 1 xylose, Stage 1 glucose, and Stage 2 xylose, respectively, at an enzyme loading of 68.4 mg/g glucan in raw switchgrass. Basis: the absolute values were recast on the basis of pretreatment of 100 g of raw Dacotah switchgrass, which contained 35.0 g glucan and 21.8 g xylan.

40–60 min for pretreatment at 140 °C. The total glucose and xylose yield curves nearly flattened out following their peak for pretreatments at 140 and 160 °C, and because of the very low xylose yields in Stage 2, total Stage 2 glucose plus xylose yields tracked glucose yields.

When looking at both Fig. 1a and b, it can be seen that the highest total Stage 1 glucose plus xylose yield did not coincide with the highest Stage 2 yield. For example, the highest Stage 1 glucose plus xylose yield at 160 °C occurred for a reaction time of 5 min, while the maximum Stage 2 glucose plus xylose yield occurred at a reaction time of 20 min. Thus, pretreatment conditions must be compromised from those favored for maximizing just Stage 1 or 2 yields to achieve the maximum combined Stage 1 and 2 yields (Lloyd and Wyman, 2005).

Fig. 1c summarizes total Stage 1 and Stage 2 glucose plus xylose yields and illustrates the partitioning of glucose and xylose between Stage 1 and 2. We can see that at 180 °C with 1 wt.% sulfuric acid, the highest glucose or xylose yield both occurred at a short pretreatment time of about 2.5 min, coincident with the highest total glucose plus xylose yield. Although glucose yields remained at a nearly steady level with further increases in time beyond the point of the maximum, the total glucose plus xylose yields dropped rapidly due to a rapid drop in xylose yields due to its degradation. A similar pattern was observed at 160 °C in that the glucose yield peaked at 20 min and remained essentially constant thereafter, while the maximum total combined glucose and xylose yield occurred at a pretreatment time of 10 min as a compromise in glucose and xylose yields. Overall, pretreatment conditions of 140 °C for a 40 min reaction time, 160 °C for 10 min, and 180 °C for 2.5 min, all with a sulfuric acid concentration of 1.0%, resulted in nearly the same maximum total glucose and xylose yields of about 86%.

3.2. Sugar yields vs. severity for dilute acid pretreatment and subsequent enzymatic hydrolysis

Fig. 2 plots the yields from a total of 45 dilute acid Stage 1 pretreatments followed by Stage 2 digestion of the residual solids at an enzyme loading of 68.4 mg protein/g glucan in raw switchgrass vs. the log of combined severity parameter. The pretreatment conditions covered a range of log CS from −0.3 to 3.0. Although many of the results for each stage follow a consistent trend, some conditions resulted in substantial scatter that departs from the overall trend. In any event, the highest combined glucose plus xylose yields of about 85% appeared at log CS of about 1.6–1.7. Several pretreatment conditions provided similar yields, with some of these tabulated in Table 2. For example, pretreatment with 1% sulfuric acid at 140 °C for 40 min (log CS = 1.6) coupled with subsequent enzymatic hydrolysis gave a total mass yield of 54.7 g glucose plus xylose (~86% theoretical yield) from 100 g raw Dacotah switchgrass at an enzyme loading of 68.4 mg/g initial glucan. Similar yields were realized at the same log CS for different combinations of acid loadings, pretreatment times, and temperatures, confirming the value of the severity parameter in correlating pretreatment data. The conditions corresponding to the highest total sugar yields in Fig. 1 all align with virtually the same value of the severity parameter and reinforce the heuristic that the yield will remain virtually constant if the reaction time is cut in half for every 10 °C increase in temperature or doubling of acid concentration.

High yields of sugars from cellulose and hemicellulose in lignocellulosic biomass through the combined operations of pretreatment and enzymatic hydrolysis are essential for commercial production of fuels or chemicals (Wyman et al., 2005). However, as illustrated in Fig. 2, the pretreatment severity to maximize xylose recovery (primarily Stage 1 xylose) is not the same as the best severity for the highest glucose yields (primarily Stage 2 glucose) from subsequent enzymatic hydrolysis of the pretreated cellulose. Our data showed that Stage 1 xylose yields peaked at a log CS of about 1.3 while a much higher log CS of 2.0 maximized Stage 2 glucose yields, an observation in line with previous studies (Lloyd

Table 2
Stage 1 and 2 mass yields of glucose and xylose following dilute acid pretreatment of Dacotah switchgrass at selected conditions followed by enzymatic hydrolysis of the pretreated solids for an enzyme loading of 68.4 mg/g glucan in raw switchgrass.

Temp (°C)	Acid conc. (%)	Pretreatment time/min	log combined severity	Stage 1 (pretreatment)			Stage 2 (enzymatic hydrolysis)			Stage 1 + 2		
				Glucose	Xylose	Total	Glucose	Xylose	Total	Glucose	Xylose	Total
140	1	10	1	2.3	20.0	22.3	21.4	2.4	23.8	23.7	22.4	46.1
140	1	40	1.6	3.2	21.2	24.4	28.8	1.5	30.3	32.0	22.7	54.7
140	1	60	1.8	3.4	20.2	23.6	30.1	1.1	31.2	33.5	21.3	54.8
160	1	10	1.7	3.4	20.0	23.4	31.4	0.0	31.4	34.8	20.0	54.8
180	1	1	1.4	3.6	11.5	15.1	31.3	0.0	31.3	34.9	11.5	46.4
180	1	2.5	1.7	3.6	20.8	24.4	30.7	0.0	30.7	34.3	20.8	55.1
180	1	5	2.4	4.8	9.8	14.6	30.3	0.0	30.3	35.1	9.8	44.9
140	0.5	40	1.1	1.9	14.5	16.4	22.4	1.9	24.3	24.3	16.4	40.7
140	0.5	80	1.4	2.7	20.0	22.7	27.4	1.7	29.1	30.1	21.7	51.8
160	2	2	1.4	2.4	11.1	13.5	28.7	0.5	29.2	31.1	11.6	42.7
160	2	5	1.7	4.0	19.1	23.1	32.4	0.1	32.5	36.4	19.2	55.6
160	2	10	2.1	3.8	12.0	15.8	31.9	0.0	31.9	35.7	12.0	47.7
180	2	10	2.7	13.9	3.0	16.9	14.8	0.0	14.8	28.7	3.0	31.7

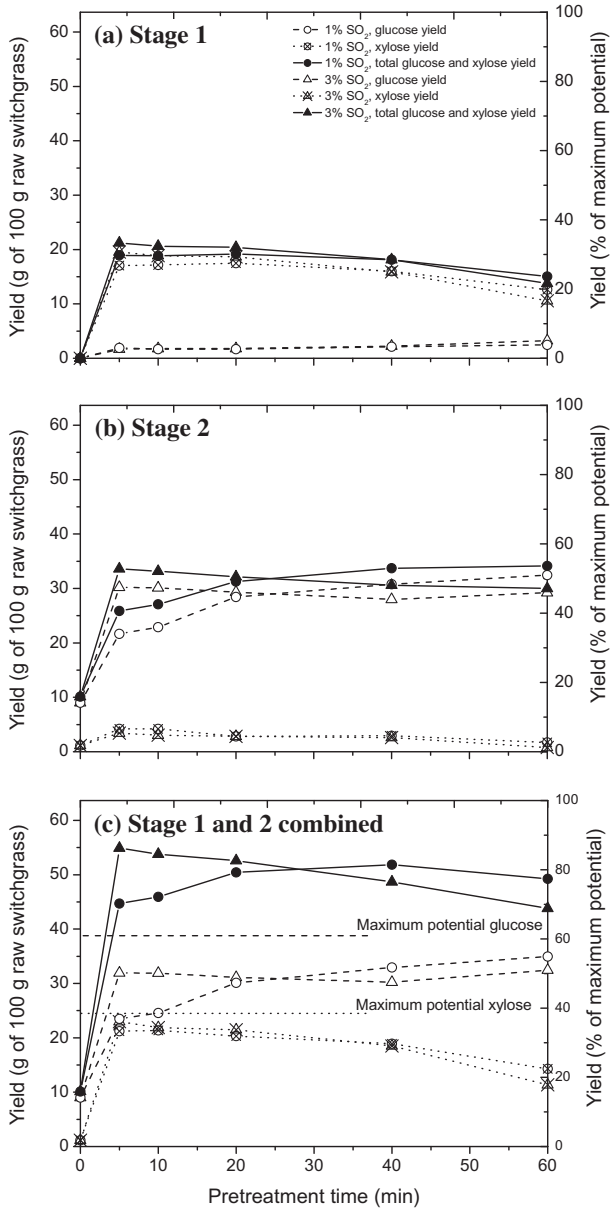

Fig. 3. (a) Stage 1 glucose and xylose yields for Dacotah switchgrass pretreated with 1% and 3% sulfur dioxide for different temperatures and times; (b) Stage 2 glucose and xylose yields for enzymatic hydrolysis of Dacotah switchgrass pretreated with 1% and 3% sulfur dioxide for different temperatures and times; (c) Total glucose plus xylose yields from combined Stage 1 and 2 for Dacotah switchgrass pretreated with 1% and 3% sulfur dioxide for different temperatures and times at an enzyme loading of 88.0 mg/g glucan in raw switchgrass.

and Wyman, 2005). A two-step steam pretreatment, with the first at low severity to hydrolyze hemicellulose and the second step at higher severity to enhance the digestibility of the solids from the first pretreatment step, can result in higher overall sugar yields than a one-step steam pretreatment process (Soderstrom et al., 2002). However, high capital and energy requirements along with an additional solid/liquid separation process between the two pretreatment steps limit the commercial viability of such a two-step pretreatment system (Wingren et al., 2004). Thus, compromise is generally required to obtain the highest possible yields from the combined operations for one step pretreatment. Although many only considered xylose release during pretreatment and glucose release during enzymatic hydrolysis, an important realization is that the release of some glucose during pretreatment and some xylose during enzymatic hydrolysis should also be factored in when defining optimal operating conditions, as shown in Fig. 2, consistent with previous findings (Lloyd and Wyman, 2005).

3.3. Sugar yields from sulfur dioxide pretreatment and subsequent enzymatic hydrolysis

Fig. 3 presents sugar yields from Stage 1, Stage 2, and combined Stage 1 and 2 operations with both 1% and 3% sulfur dioxide loadings over a range of times. In the same manner as for dilute sulfuric acid pretreatment, xylose was the primary sugar released during Stage 1 pretreatment, with peak mass yields of 17.8 and 19.5 g for 5–10 min reaction times with 1% and 3% sulfur dioxide loadings, respectively (Fig. 3a). Xylose yields decreased slowly with reaction time longer than 20 min indicating the rate of xylose degradation was faster than the rate of release during this period. On the other hand, glucose yields continually increased with time in Stage 1, similar to the results with dilute acid. When the trends are combined, the maximum total glucose and xylose yields in Stage 1 occurred at 5–10 min.

Fig. 3b shows that glucose dominated Stage 2 sugar yields during enzymatic hydrolysis of sulfur dioxide pretreated switchgrass performed at Stage 1 conditions. For 3% SO_2 loading, the maximum Stage 2 glucose plus xylose mass yield (mainly glucose) of 33.2 g/100 g raw biomass occurred over a reaction time span of 5–10 min and stayed flat for longer times. Although a similarly high Stage 2 sugar mass yield of 33.5 g/100 g raw biomass was achieved for pretreatment with 1% sulfur dioxide, a longer reaction time of 40–60 min was required.

When examining combined Stage 1 and 2 sugar yields in Fig. 3c, a total mass yield of 54 g glucose plus xylose (85%) was realized from Dacotah switchgrass with a 3% sulfur dioxide loading and a reaction time of 5–10 min. Pretreatment with 1% sulfur dioxide gave a slightly lower total mass yield of 51 g (81%) at a longer reaction time of 40 min, as also shown in Fig. 3c.

We further tested a range of 1–10% sulfur dioxide loadings at 180 °C for 10 min, with the sugar yield data tabulated in Table 3. Compared to water-only pretreatment (0% sulfur dioxide loading), adding sulfur dioxide significantly improved both Stage 1 and Stage 2 sugar yields, consistent with the previous studies on corn stover (Clark et al., 1989). Furthermore, higher sulfur dioxide loadings led to greater xylose recovery in Stage 1 and higher glucose yields from Stage 2 indicating significant hemicellulose removal and improvement in digestibility for higher sulfur dioxide concentrations. For instance, among the conditions tested, a 5–10% sulfur dioxide loading resulted in the highest total glucose plus xylose mass yields of about 56 g/100 g raw switchgrass. This result is in line with previous studies showing that increasing SO_2 concentration increased monomeric sugar yield from corn stover (Clark et al., 1989). In addition, xylose degradation in Stage 1 was not significant even at high sulfur dioxide loadings with a high log CS of 2.1, although it is well established that degradation of monomeric sugars increases at high pretreatment severities (Chum et al., 1990). Thus, sulfur dioxide appears to be less powerful than dilute sulfuric acid in catalyzing sugar degradation, resulting in higher sugar yields over a broader range of pretreatment severities. This outcome may be due to the higher pH for sulfur dioxide compared to dilute sulfuric acid not favoring dehydration of xylose to furfural while still being effective in hydrolyzing hemicellulose to sugars (Lloyd and Wyman, 2003).

3.4. Sugar yields vs. severity for sulfur dioxide pretreatment and subsequent enzymatic hydrolysis

Fig. 4 plots sugar yield data from all sulfur dioxide pretreatments followed by digestion of the cellulose in the residual solids

Table 3
Stage 1 and 2 mass yields of glucose and xylose following sulfur dioxide pretreatment of Dacotah switchgrass at selected conditions followed by enzymatic hydrolysis of the pretreated solids for an enzyme loading of 88.0 mg/g glucan in raw switchgrass.

Temp (°C)	SO_2 loading (%)	Pretreatment time/min	log combined severity	Stage 1 (pretreatment)			Stage 2 (enzymatic hydrolysis)			Stage 1 + 2		
				Glucose	Xylose	Total	Glucose	Xylose	Total	Glucose	Xylose	Total
180.0	0.0	10.0	−0.7	2.3	8.1	10.4	11.6	4.9	16.5	13.9	13.0	26.9
180.0	1.0	10.0	0.7	2.7	17.3	20.0	21.8	4.0	25.8	24.5	21.3	45.8
180.0	3.0	10.0	1.0	2.8	18.9	21.7	30.1	2.9	33.0	32.9	21.8	54.7
180.0	5.0	10.0	1.7	3.0	19.2	22.2	31.7	2.2	33.9	34.7	21.4	56.1
180.0	10.0	10.0	2.0	3.3	19.3	22.6	32.2	1.7	33.9	35.5	21.0	56.5

at an enzyme loading of 88 mg protein/g glucan in the raw switchgrass vs. the log CS parameter over a range of −0.7–2.3. The highest total Stage 1 plus Stage 2 glucose and xylose yields of about 85% appeared at a log CS of about 1.7. However, similar to dilute acid pretreatment, the maximum Stage 1 xylose yield occurred at less severe pretreatment conditions compared to the severity required to maximize the Stage 2 glucose yield. These results also show that pretreatment conditions and corresponding sugar yields for switchgrass were comparable to those for sulfur dioxide pretreatment of corn stover, hardwood, and sugarcane bagasse (Table 1). However, softwood appears to require slightly more severe conditions to achieve high sugar yields (Bura et al., 2002; De Bari et al., 2007; Martin et al., 2002; Ramos et al., 1992; Stenberg et al., 1998).

3.5. Effect of enzyme loading on digestibility

Although high yields can be realized by applying high enzyme loadings following biomass pretreatment, enzyme doses need to be significantly reduced to make the conversion process commercially attractive (Wyman, 2007), and pretreatment conditions and subsequent enzymatic hydrolysis must be optimized for maximum sugar release with the lowest possible amount of enzyme. Therefore, we investigated effects of enzyme loadings on enzymatic hydrolysis for selected conditions for dilute acid and sulfur dioxide pretreatment of Dacotah switchgrass. As summarized in Table 4, for one of the best dilute acid pretreatment conditions (1% dilute sulfuric acid, 140 °C, 40 min (log CS = 1.6)), a mass yield of 57.3 g glucose plus xylose (~90% of maximum potential) from 100 g raw Dacotah switchgrass was obtained at an enzyme loading of 96.6 mg/g glucan in raw switchgrass. Similarly, a mass yield of about 56.6 g glucose plus xylose (~89% of maximum potential) was achieved for the best SO_2 pretreatment conditions (5% sulfur dioxide, 180 °C, 10 min (log CS = 1.7)) at the same high enzyme loading. However, at lower enzyme loadings, selected SO_2 pretreatments led to higher sugar yield than dilute acid pretreatment. For example, a mass yield of about 48.7 g glucose plus xylose (~76% of maximum potential) was achieved for selected sulfur dioxide pretreatment conditions at a low enzyme loading of 4.8 mg/g glucan in raw switchgrass, while at the same enzyme loading, dilute acid pretreatment gave a mass yield of only 44.4 g (70% of maximum potential). However, further investigations are needed to define enzyme formulations and loadings to maximize total sugar yields at lower protein loadings. For example, supplementation of cellulases with beta-glucosidase, pectinase, and xylanase may potentially improve sugar yields with low enzyme loadings in Stage 2 (Berlin et al., 2007; Kumar and Wyman, 2009).

This study showed nearly identical sugar yields for sulfur dioxide and sulfuric acid pretreatments at optimal conditions. The main advantage of using sulfur dioxide is that impregnation with gaseous sulfur dioxide is more rapid and uniform than for sulfur dioxide in cellulosic biomass, especially for large scale applications (Zhu et al., 2009). A potential problem associated with sulfur dioxide is somewhat higher amounts of xylooligomers in the pre-

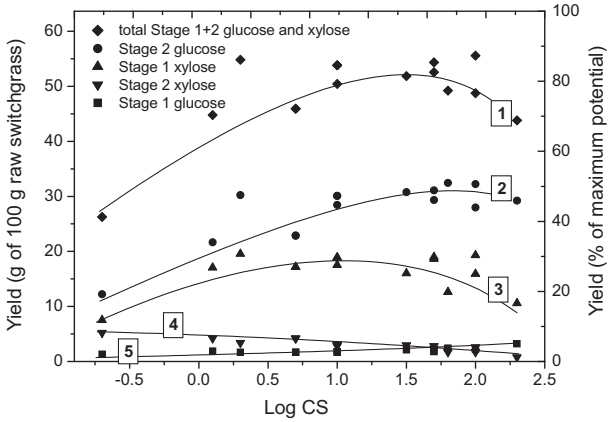

Fig. 4. Glucose and xylose yields plotted against the log combined severity (log CS) parameter for all temperatures and times applied for sulfur dioxide pretreatment and subsequent enzymatic hydrolysis of Dacotah switchgrass with lines reflecting approximate data trends. Trendlines 1–5 represent total Stage 1 + 2 glucose plus xylose, Stage 2 glucose, Stage 1 xylose, Stage 1 glucose, and Stage 2 xylose, respectively at an enzyme loading of 88.0 mg/g glucan in raw switchgrass.

treatment hydrolysate at low sulfur dioxide loadings. Because xylooligomers are strong inhibitors to enzymatic hydrolysis of cellulose, a secondary hydrolysis step may be required to breakdown xylooligomers into monomers to mitigate inhibition (Qing et al., 2010). In addition, many organisms cannot ferment oligomers, making hydrolysis essential to high final product yields. Furthermore, sulfur dioxide is more expensive than sulfuric acid, and SO_2 requires more careful handling to address safety and environmental concerns. Sulfur dioxide also lends itself to recycling better than sulfuric acid, although at a cost. For both SO_2 and H_2SO_4 pretreatments, lignin may be recovered for use as a chemical or burned to provide heat and power. Lignin sulfonates resulting from SO_2 pretreatment could have many potential applications such as a surface stabilizer, tanning agent, ingredient in adhesives, and lubricants, with a few established in the paper pulping industry (Stewart, 2008).

3.6. Material balances at conditions giving high sugar yields

Fig. 5 summarizes material balances for the pretreatment conditions that realized the highest total sugar yields for dilute sulfuric acid and sulfur dioxide pretreatments. On the basis of 100 g of raw switchgrass, about 54–60 g of pretreated solids can be recovered after pretreatment. On the same basis, about 24.3 and 22.2 g of glucose plus xylose and their oligomers can be recovered following post-hydrolysis of the pretreatment hydrolyzate for dilute acid and sulfur dioxide pretreatment, respectively. Furthermore, about 30.1 and 33.4 g of glucose plus xylose can be recovered from enzymatic hydrolysis of dilute acid and sulfur dioxide pretreated

Table 4
Effect of enzyme loading on sugar yields for dilute acid and SO_2 pretreatments of Dacotah switchgrass at selected conditions.

Pretreatment conditions	Enzyme loading mg/g	Stage 1 (Pretreatment)			Stage 2 (enzymatic hydrolysis)			Stage 1 + 2		
		Glucose	Xylose	Total	Glucose	Xylose	Total	Glucose	Xylose	Total
Dilute acid pretreatment: 140 °C, 1% H_2SO_4, 40 min, log CS = 1.6	4.8	3.2	21.2	24.4	18.9	0.9	19.8	22.1	22.1	44.4
	24.2	3.2	21.2	24.4	26.8	1.3	28.1	30.0	22.5	52.5
	48.3	3.2	21.2	24.4	28.6	1.5	30.1	31.8	22.7	54.5
	96.6	3.2	21.2	24.4	31.0	1.9	32.9	34.2	23.1	57.3
SO_2 pretreatment: 180 °C, 5% SO_2, 10 min, log CS = 1.7	4.8	3.0	19.2	22.2	24.8	1.7	26.5	27.8	20.9	48.7
	24.2	3.0	19.2	22.2	31.0	2.1	33.1	34.0	21.3	55.3
	48.3	3.0	19.2	22.2	31.2	2.2	33.4	34.2	21.4	55.6
	96.6	3.0	19.2	22.2	32.0	2.4	34.1	35.0	21.6	56.6

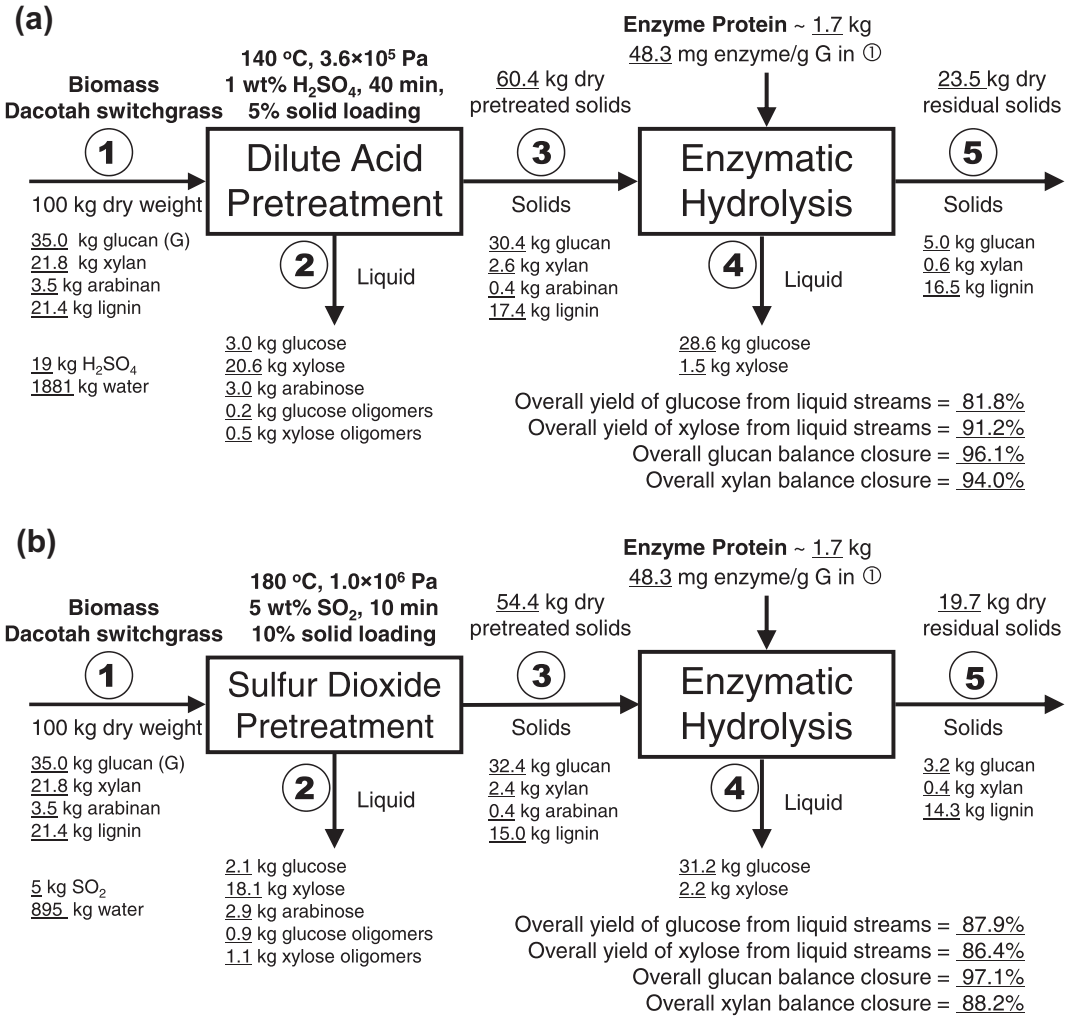

Fig. 5. Material balances for (a) dilute acid pretreatments at 140 °C with 1.0% acid for 40 min and (b) sulfur dioxide pretreatment at 180 °C with 5.0% SO_2 for 10 min. Both include yields of glucose and xylose for enzymatic hydrolysis of the washed pretreated solids with 48.3 mg protein/g glucan in raw switchgrass for 72 h.

switchgrass for the conditions selected, respectively. Although the material balance indicated loss of some mass during pretreatment and enzymatic hydrolysis, the overall sugar recovery in the liquid streams was over 85%, confirming that selected pretreatment conditions can preserve most of the sugars and substantially enhanced the effectiveness of enzymatic hydrolysis. The overall glucan closure of 96.1–97.1% was higher than that of xylan of 88.2–94.0%, and is due to the greater chemical robustness of glucose, in agreement with previous literature (Lloyd and Wyman, 2005). On average, about 20 g of the feedstock could not be accounted for and may be due to either lost as gaseous compounds or conversion to some compounds not determined in this study such as furfural, 5-HMF, and Humins. During pretreatment and enzymatic hydrolysis, lignin solubilization into the liquid stream may also contribute to loss of total mass. Thus, additional study is needed to fully account for all components, particularly if the data is to be used to support a commercial project.

4. Conclusions

Sugar yields from switchgrass pretreatment and enzymatic hydrolysis of pretreated solids were sensitive to pretreatment con-

ditions, and compromises were needed to optimize overall process yields. For dilute acid pretreatment, time–temperature combinations that resulted in a log CS of 1.6 gave a mass yield of about 54.5 g glucose plus xylose from 100 g raw Dacotah switchgrass at an enzyme loading of 48.3 mg/g initial glucan while similar yields were realized with sulfur dioxide pretreatment at a log CS of 1.7. However, sugar yields of sulfur dioxide pretreatment were higher than for dilute acid pretreatment at lower enzyme loadings.

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