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Comparison of microwaves to fluidized sand baths for heating tubular reactors for hydrothermal and dilute acid batch pretreatment of corn stover

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ABSTRACT

Heating of batch tubular reactors with fluidized sand baths and with microwaves resulted in distinctive sugar yield profiles from pretreatment and subsequent enzymatic hydrolysis of corn stover at the same time, temperature, and dilute sulfuric acid concentration combinations and hydrothermal pretreatment conditions. Microwave heated pretreatment led to faster xylan, lignin, and acetyl removal as well as earlier xylan degradation than sand baths, but maximum sugar recoveries were similar. Solid state CP/MAS NMR revealed that microwave heating was more effective in altering cellulose structural features especially in breakdown of amorphous regions of corn stover than sand bath heating. Enzymatic hydrolysis of pretreated corn stover was improved by microwave heating compared to sand bath heating. Mechanisms were proposed to explain the differences in results for the two systems and provide new insights into pretreatment that can help advance this technology.

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1. Introduction

A pretreatment step is essential to overcome the natural recalcitrance of lignocellulosic biomass to enzymatic hydrolysis to sugars through opening up the lignocellulosics complex and making high sugar yields possible (Mosier et al., 2005). Some studies have shown that pretreatment is one of the most expensive steps in biologic conversion of cellulosic biomass and advanced pretreatment technologies are needed to significantly reduce costs, improve cellulose digestibility, simplify upstream and downstream operations, and provide revenues from co-products (Yang and Wyman, 2008). Hydrothermal and dilute acid pretreatment have proven to be promising technologies. When just water or steam are used to heat biomass to around 180–220 °C via the hydrothermal or autohydrolysis approach, acetic acid released from acetylated hemicellulose has been credited with catalyzing hydrolysis of hemicellulose and some of the cellulose into soluble oligomers and monomeric sugars (Mosier et al., 2005). However, dilute acid pretreatment with 1–5% sulfuric acid significantly enhances sugar yields compared to the hydrothermal approach (Grethlein, 1980). Both approaches as well as most of the other leading pretreatment options rely on heating biomass to temperature between about 140 and 220 °C, with laboratory operations typically using convection- or conduction-based heating (Jacobsen and Wyman, 2001; Stuhler and Wyman, 2003; Viamajala et al., 2010), with short heat-up and cool down times desired to facilitate understanding the reaction kinetics and assure consistent kinetic results and interpretation.

Microwaves are electromagnetic waves spanning a frequency range from 300 MHz (3×10^8 cycles/s) to 300 GHz (3×10^{11} cycles/s), with most industrial and household microwave processing operating at a frequency of 2.45 GHz. Microwave technology was developed in 1940's driven by military needs and was later introduced into civilian markets. Over the decades, this technology has spread widely to such applications as food processing, analytical chemistry, heating and vulcanization of rubber, plasma processing, chemical synthesis and processing, and waste remediation (Clark et al., 2000). Compared with conduction/convection heating, which is based on intramolecular heat transfer, microwave heats the target object directly by applying an electromagnetic field to dielectric molecules, such as water, causing vibration and rotation that releases heat (Newnham et al., 1991). Thermal and non-thermal effects have been proposed to explain the interaction of microwaves with processed materials, with the former primarily due to absorption of microwave energy and dipolar reorientation with the imposed electric field (Newnham et al., 1991). Microwave heating can, therefore, selectively heat polar polymers in mixtures and may target compounds with high dielectric loss and high polarity preferentially and this characteristic has been shown to enhance a number of chemical processes, including catalytic reactions

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utilizing metallic or dielectric catalysts, gas-phase synthesis of metal halides and nitrides, and metal reduction processes (Bond et al., 1993). In addition to the thermal effect, nonthermal "microwave effects", including accelerated apparent kinetics, retarded kinetics, and dependencies of the heating rate and structure of the cured polymer structures formed at the pulse repetition frequency have been reported (Lewis et al., 1992; Bond et al., 1993). The most prevalent reports of microwave effects have been acceleration of reaction rates, with a proposed mechanism for the "speed-up effect" in polymers being non-equilibrium, non-uniform energy distribution at the molecular level, resulting in some dipoles having greater energy than the "average" energy of adjacent groups (Lewis et al., 1992). As reported previously, the decomposition rate of many difficult-to-dissolve materials is greatly enhanced by microwaves, leading to increases in reaction rate by 10–100 times (de la Hoz et al., 2005). The deep penetration of microwaves into aqueous environments could make microwave heating appealing for pretreatment of cellulosic biomass by avoiding the temperature gradients typical for conductive methods (Jacobsen and Wyman, 2001; Stuhler and Wyman, 2003) and overcoming the limitations on solids concentrations for mixed reactors (Yang and Wyman, 2008).

Ooshima et al. (1984) and Azuma et al. (1985) first introduced microwave heating for processing cellulosic biomass and demonstrated enhanced cellulose saccharification following microwave treatment. As summarized in Table 1, several studies confirmed enhanced sugar yields following microwave heating of pretreatments with just water or with added alkali or acid on various lignocellulosic biomass materials including barley husk, rice straw, sugar cane bagasse, softwood and switchgrass. For example, Zhu et al. (2005) reported a 65% glucose yield and 78% total carbohydrate conversion from rice straw using microwave heated alkaline pretreatment. An even more recent study (Keshwani and Cheng, 2010) reported 80–85% sugar yields from switchgrass using dilute sodium hydroxide solutions with microwave heating and suggested that the dielectric loss tangent of the alkali solution partially contributed to pretreatment severity.

Although many hypotheses have been advanced to explain how microwave heating pretreatment improves biomass digestibility, the mechanism is not yet fully established. Some previous studies "boiled" biomass suspension in an "open air" beaker and consequently did not control water losses to evaporation, while other studies applied a constant microwave power level without controlling pretreatment temperature. To gain better insight into the mechanism of microwave heating pretreatment, pretreatment temperature and moisture levels must be carefully controlled and material balances need to be closed to assure accurate data for microwave heating. In addition, a side by side comparison of microwave heating to conventional heating, such as with a sand bath, has been rarely reported (Hu and Wen, 2008), and consideration of sugar release kinetics and analysis of the compositional and structural modification that can provide valuable insight into the mechanism by which microwave heating acts have not been included. Thus, this study aimed at developing a systematic comparison of microwave heating to heating by a conventional sand bath approach for application to hydrothermal and dilute acid pretreatment of biomass. Sugar release in pretreatment and subsequent enzymatic hydrolysis of corn stover was tracked for application of each method at well-controlled conditions, and sugar balances were determined to validate the treatment efficiency.

Solid state NMR has proven to be a useful tool for structural investigation on lignocellulosic biomass at a molecular level. It is a nondestructive method that can be employed for direct characterization of the biomass without the need to isolate or fractionate the polymeric components of biomass (Maunu, 2002). It has been used for studying the changes in the chemical structures of oak wood (Vane et al., 2003) and wheat bran (Locci et al., 2008) induced by fungal treatment, and for monitoring morphological changes in cellulose of Lespedeza stalks during steam explosion (Wang et al., 2009). Therefore, solid state CP/MAS NMR was applied to follow compositional changes and alteration in cellulose structural features for correlation to digestibility that can help elucidate differences between sand bath heating and microwave irradiation in deconstructing cellulosic biomass.

2. Methods

2.1. Biomass feedstock

Raw Kramer corn stover milled to quarter inch sized particles was generously provided by the National Renewable Energy Laboratory (NREL, Golden, CO) from their source at the Kramer farm in Wray, CO. The moisture content of the corn stover was about 6%. Upon receipt, it was sealed in air-tight bags and stored at −20 °C until use. The composition of raw corn stover was measured as described in Section 2.5.

2.2. Pretreatment

For batch tube pretreatment, approximately 10 ml of a 5% solids loading of corn stover that had been presoaked in either just water or a 1 wt.% sulfuric acid solution was added to ½ inch diameter by

Table 1
Summary of literature reports on microwave pretreatment of cellulosic biomass.[a]

Feedstock	Microwave device	Chemicals uses	Temperature and time	Microwave power	Sugar yields	References
Barley husk	MLS-1200 Mega Microwave workstation, Milestone, Sorisole, Italy	0.5% H_2SO_4 or water-only	200 °C for 5 min or 210 °C for 10 min	N/A	G: 85%, X: 46%	Palmarola-Adrados et al. (2005)
Rice straw	WD700 (MG-5062T) type domestic microwave	1% NaOH	Temperature uncontrolled, 15 min to 2 h	Fixed at 300–700 W	G: 65%; total sugar conversion: 78%	Zhu et al. (2005)
Rice straw and sugar cane bagasse	Turbora Model TRX-1963 domestic microwave	Glycerin in water solution	Temperature uncontrolled, 10 min	Fixed at 240 W	N/A	Ooshima et al. (1984) and Kitchaiya et al. (2003)
Softwoods	Toshiba Model TMB 3210	Water-only	219–226 °C for (estimated with an infra-red thermometer)	Up to 2.4 kW	88–93% total sugar	Azuma et al. (1985)
Switchgrass	Customized Sharp/R-21 HT domestic microwave	0.1 g NaOH/g biomass	190 °C, 30 min	Up to 1 kW	G + X: 99%	Hu and Wen (2008)
Switchgrass	Panasonic Corporation, model NN-S954	1–3% NaOH	Temperature uncontrolled, 5–20 min.	Fixed at 250 W	80–85% total sugar	Keshwani and Cheng (2010)

[a] G: glucose; X: xylose; N/A: not available.

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6 inch long Hastelloy tube reactors (Yang and Wyman, 2009). The tubes were then sealed by Teflon plugs and stainless steel caps. Next, the sealed tubes were heated in a sand bath for residence times of 5, 10, 20, and 40 min at 180 °C for hydrothermal pretreatment or over the same time periods at 140 °C for pretreatment with dilute acid.

For microwave pretreatment, all reactions were performed in the CEM Discover™ unit graciously provided by CEM Corporation, Mathews, NC. Approximately 10 ml of presoaked corn stover was added to sealed glass vessels by an otherwise identical approach as described for adding corn stover to the tube reactors. The vessels were sealed with Teflon coated caps and placed in the CEM monomode microwave device by a robot and irradiated for a set temperature and time. Temperature (°C), irradiation power (Watts), time (min), and pressure (kPa) were monitored and controlled by Synergy software. The average microwave power input for maintaining the target pretreatment temperature was 26 W at 140 °C and 75 W at 180 °C.

After pretreatment, the reactors were quenched by quickly transferring them to a room temperature water bath until the temperature dropped to 30 °C within around 1–2 min. The pretreated slurry was immediately filtered using filter paper (Whatman GF/F-pore size 0.7 μm, Piscataway, NJ) to separate solids from liquid. The hydrolyzate was collected and stored at 4 °C for further analysis. The collected solids were washed with room temperature DI water (10 times of raw biomass weight).

2.3. Enzymes

Spezyme CP cellulase (lot# 301-04075-034; 58.2 FPU/ml, 123 mg protein/ml) enzyme was generously provided by the Genencor Division of Danisco US, Inc. (Rochester, NY). Novozyme188 β-glucosidase (batch# 097K0682; 665 CBU/ml, 140 mg protein/ml) was purchased from Sigma–Aldrich Chemicals.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was performed according to NREL Laboratory Analytical Procedure (LAP) 009 in duplicates at 50 °C in 125 mL Erlenmeyer flasks containing 1% glucan concentrations of pretreated corn stover in 0.05 M citrate buffer (pH 4.8) with 10 mg/L sodium azide using a thermostatic shaker air bath set at 150 rpm (Selig et al., 2008). Substrate blanks without enzyme and enzyme blanks without substrate were run in parallel. Digestibility was determined at cellulase plus β-glucosidase loadings of 50 mg of protein/g glucan plus xylan in the original biomass (corresponding to around 30 FPU/g glucan in pretreated solids) supplemented with β-glucosidase at a Novozyme 188 to Spezyme CP ratio of 0.08:1 v/v (equivalent to CBU to FPU activity ratio of 1:1), unless otherwise stated. 700 μl samples taken at 72 h were filtered through 0.2 μm nylon filter vials (Alltech Associates, Inc., Deerfield, IL) and kept refrigerated at 4 °C or frozen at −20 °C until analyzed. Sugar concentrations 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 de-ashing cartridges (Catalog No. 125-0119, Bio-Rad Labs, Richmond, CA, USA).

2.5. Compositional and sugar analysis

Glucan, xylan, and Klason-lignin contents of the untreated corn stover and pretreated solids were measured following standard NREL LAPs "preparation of samples for compositional analysis" and "determination of structural carbohydrates and lignin in biomass" (Hames et al., 2008; Sluiter et al., 2008a). Without further mention, lignin refers to Klason-lignin in this paper. Glucose and xylose concentrations of hydrolyzates from pretreatment and enzymatic hydrolysis were quantified by HPLC as described in Section 2.4. Furthermore, part of the hydrolyzates from pretreatment was post-hydrolyzed according to standard NREL LAPs to obtain a solution with only monomeric sugars, which was used to calculate the monomer equivalents of both mono- and oligomeric-sugars in pretreatment hydrolysate (Sluiter et al., 2008a). Concentrations of acetic acid and furfural in pretreatment hydrolyzates were quantified by following the standard NREL LAPs "determination of sugars, byproducts, and degradation products in liquid fraction process samples" (Sluiter et al., 2008b).

2.6. Sugar yield calculations

For clarity in reporting these results, the terms Stage 1 and Stage 2 refer to the pretreatment step of corn stover and enzymatic hydrolysis step of the pretreated solids, respectively, in this paper. All yields were reported in four ways: (1) sugar mass yields on the basis of 100 g of raw corn stover to provide a perspective on the sugar release from that feedstock, (2) xylose percent yields compared to the maximum possible from the total xylan in corn stover, (3) glucose percent yields compared to the maximum possible from the total glucan in the raw corn stover, and (4) glucose plus xylose percent yields compared to the total glucose plus xylose in raw corn stover. The latter definition was applied to provide a perspective on the relative contribution of each sugar to total sugar production. Thus, based on the original feedstock composition of 35.2% glucan and 20.6% xylan, the maximum potential mass yield of glucose and xylose on their monomeric sugar basis was 39.1 and 23.4 g, respectively, per 100 g of raw corn stover giving a combined total of 62.5 g sugar per 100 g of raw feedstock.

Mass and percent yields of glucose and xylose were reported for the two stages separately, i.e., Stage 1 glucose, Stage 1 xylose, total Stage 1 glucose plus xylose or "total sugars," Stage 2 glucose, Stage 2 xylose, and Stage 2 total sugars. Yields from the two stages combined were expressed as combined Stages 1 + 2 glucose, combined Stages 1 + 2 xylose, and combined Stages 1 + 2 sugar (i.e., glucose plus xylose). Thus, the word "sugar" is applied to describe the total of glucose plus xylose while the word "combined" refer to the total yields from the two stages. Furthermore, yields of glucose and xylose included the total amount of monomers and oligomers together expressed as monomeric sugar equivalents. For instance, the Stage 1 xylose yield included both the xylose and xylooligomers converted to xylose equivalents released into the pretreatment hydrolysate during pretreatment compared to the maximum total xylose.

2.7. Solid state CP/MAS NMR

Solid state CP/MAS ^{13}C NMR experiments were performed with a Bruker Avance-400 spectrometer operating at 100.59 MHz for ^{13}C in a Bruker double-resonance 4-mm MAS probe head. The samples (~50% moisture) were packed in a 4-mm cylindrical Zirconia rotor fitted with Kel-F caps and spun at the magic angle with a speed of 8.0 kHz. Spectra were acquired with the following parameters: 4.5-μs pulse length (90° proton pulse), 2.0-ms contact time, 5-s recycle delay, 0.034-s acquisition time, and 2048 data points. Typically 8129 scans were accumulated for each sample with high-power ^{1}H decoupling. All spectra were obtained at ambient temperature. Adamantine was used for setting the Hartman–Hahn matching conditions. Chemical shifts were calibrated using an external sample of glycine with the maximum intensity of the carbonyl carbon resonance assigned to 176.03 ppm (Hult et al., 2002).

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3. Results and discussion

3.1. Temperature profiles for microwave and sand bath heating

Although microwave (MW) and sand bath (SB) heating modes are much different, they displayed similar heat up patterns when applied to reaction tubes at the conditions applied in this study, as summarized in Table 2. The times to reach 140 and 180 °C were about 2 and 3 min, respectively, for either microwaves or the sand bath. In addition, the reaction temperature was controlled within ±2 °C for both heating approaches.

3.2. Composition of pretreated corn stover

As shown in Table 3, the raw corn stover employed for this study contained about 35.2% glucan, 20.6% xylan, and 22.6% lignin. The remains are other components (e.g. arabinan, galactan, mannan, acetyl groups, protein, ash etc.) which are not quantified in this research but have been reported elsewhere (Kumar et al., 2009). In addition, Table 3 shows the amounts of lignin, glucan, and xylan components left in the pretreated feedstock solids on the basis of the original 100 g of dry feedstock for hydrothermal and dilute acid pretreatments using microwave and sand bath heating. Table 3 also presents the percentages of each major component that were removed from corn stover at each condition, as calculated by mass balances on each. As expected, the most prominent compositional change caused by both pretreatments was a decrease in xylan content. For instance, from the original 20.6 g xylan/100 g raw corn stover, the solids resulting from sand bath (SB) heated hydrothermal pretreatment contained 6.2–17.1 g xylan/100 g raw corn stover, with lower xylan contents resulting from longer pretreatment times. Microwave (MW) heating showed even greater xylan removal to about 1.8–15.9 g xylan/100 g raw corn stover for hydrothermal pretreatment over the same time span. Dilute acid enhanced xylan removal and resulted in a xylan content of 2.1–6.1 g/100 g raw corn stover and 1.0–6.1 g/100 g raw corn stover heated by SB and MW, respectively. In summary, microwave heating reduced xylan contents more than sand bath heating at identical pretreatment conditions (i.e., temperature, time) as a result of greater xylan reaction. In line with compositional data, the percent xylan removal on basis of mass balance also verified that MW heating resulted in faster and greater xylan removal for both hydrothermal and dilute acid pretreatment.

Compared to the 22.6 g lignin in100 g of untreated corn stover, the lignin left in the pretreated solids ranged from 17.4 to 20.7 g per 100 g raw corn stover, suggesting that lignin removal was moderate by hydrothermal and dilute acid pretreatment for both heating options. However, dilute acid pretreatment removed more lignin than hydrothermal pretreatment, and microwave heating removed slightly more lignin than sand bath heating at identical conditions. The mass balance data show that MW heating removed more lignin for both hydrothermal and dilute acid pretreatment at any pretreatment time.

The glucan left in the hydrothermally pretreated solids ranged from 31.0 to 34.4 g per 100 g raw corn stover over 40 min of pretreatment, while the glucan content in the dilute acid pretreated solids was slightly lower, ranging from 29.9 to 34.1 g during this time span. However, no significant differences were seen between MW and SB heating in terms of Stage 1 glucose percent yields. Our material balances also showed that about 85–98% of the original glucan was preserved in the pretreated solids after hydrothermal and dilute acid pretreatment, respectively, over the range of pretreatment times tested.

Table 2
Characteristic features of microwave and sand bath heating when applied to pretreatment of corn stover at the same temperatures.

	140 °C		180 °C	
	Microwave	Sand bath	Microwave	Sand bath
Heat-up time, min	2.1	2.0	2.9	3.2
Average *T*, °C	140.0	140.2	180.0	180.1
Cool-down time to 80 °C, min	1.5	1.0	2.9	2.0

3.3. Sugar yields from hydrothermal pretreatment followed by enzymatic hydrolysis

Fig. 1 shows distinct difference in sugar yield patterns for SB and MW heating applied to hydrothermal pretreatment. Although

Table 3
Composition of untreated corn stover and mass of lignin (Klason lignin only), glucan, and xylan left in the solids following pretreatment at the conditions noted. Also shown in the last four columns are the amount of lignin, glucan and xylan removed and the overall recovery of solids following hydrothermal (HT) and dilute acid (DA) pretreatment of corn stover by heating with microwaves (MW) or a sand bath (SB).[a]

Substrate	Pretreatment time (min)	Mass, g/100 g raw corn stover			Lignin removal[b] (%)	Glucan removal[b] (%)	Xylan removal[b] (%)	Solid yields[b] (%)
		Lignin	Glucan	Xylan				
Untreated	–	22.6 ± 1.5	35.2 ± 3.3	20.6 ± 2.3	0	0	0	100.0
MW-HT	5	20.7 ± 0.4	33.8 ± 0.5	15.9 ± 0.2	8.4	4.0	22.8	75.9
	10	17.3 ± 0.9	33.1 ± 1.2	9.2 ± 0.8	23.5	6.0	55.3	60.5
	20	17.1 ± 0.3	32.7 ± 0.0	3.6 ± 0.1	24.3	7.1	82.5	53.8
	40	17.4 ± 0.3	31.7 ± 0.4	1.8 ± 0.0	23.0	9.9	91.3	50.4
SB-HT	5	21.1 ± 0.6	34.4 ± 1.7	17.1 ± 0.9	6.6	2.3	17.0	78.9
	10	18.7 ± 0.4	33.2 ± 0.6	13.3 ± 0.3	17.3	5.7	35.4	67.4
	20	18.5 ± 0.8	32.5 ± 1.0	9.7 ± 0.6	18.1	7.7	52.9	62.7
	40	18.0 ± 0.9	31.0 ± 1.1	6.2 ± 1.1	20.4	11.9	69.9	55.5
MW-DA	5	19.3 ± 0.0	33.8 ± 0.8	1.5 ± 0.0	14.6	4.0	92.7	55.8
	10	16.4 ± 2.1	32.7 ± 0.3	1.2 ± 0.1	27.4	7.1	94.2	55.2
	20	14.6 ± 1.1	31.7 ± 0.7	1.0 ± 0.0	35.4	9.9	95.1	51.8
	40	13.9 ± 0.3	29.9 ± 0.5	1.0 ± 0.5	38.5	15.1	95.1	48.2
SB-DA	5	20.3 ± 1.5	34.1 ± 2.4	6.1 ± 1.3	10.2	3.1	70.4	65.7
	10	17.7 ± 3.2	33.4 ± 2.0	4.7 ± 1.2	21.7	5.1	77.2	64.8
	20	16.3 ± 2.4	32.7 ± 2.2	2.8 ± 1.1	27.9	7.1	86.4	55.3
	40	14.9 ± 3.7	30.6 ± 4.2	2.1 ± 0.0	34.1	13.1	89.8	52.8

[a] All composition values are based on 100 g raw corn stover.
[b] Also shown are amounts of each major component that was removed based on mass balances.

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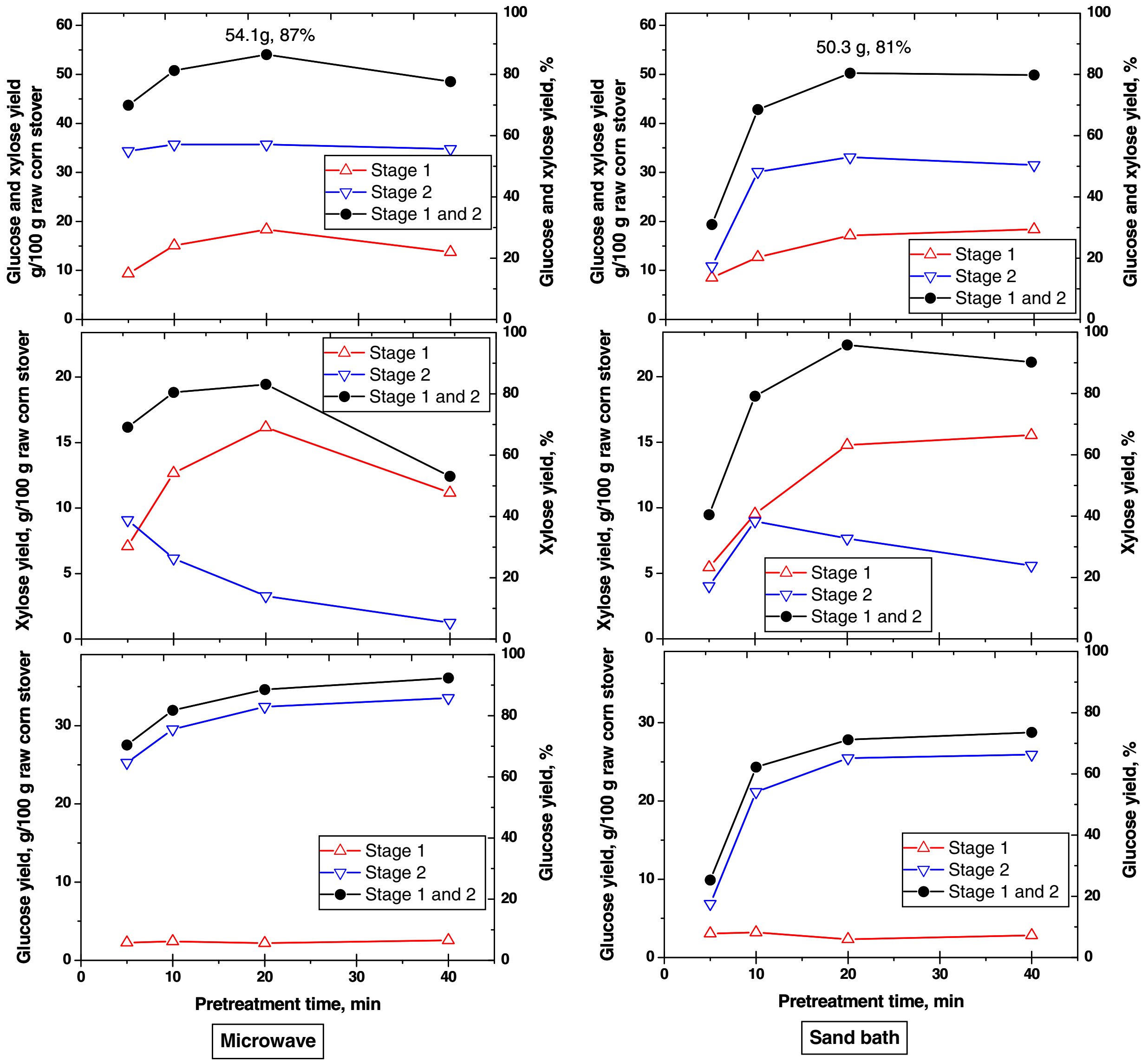

Fig. 1. Glucose, xylose, and total glucose plus xylose yields from hydrothermal pretreatment of corn stover at 180 °C and subsequent enzymatic hydrolysis of the resulting solids. Stage 1 refers to pretreatment, and Stage 2 refers to enzymatic hydrolysis. Mass sugars yields are reported on the left ordinate on the basis of 100 g of corn stover being processed, dry basis, and percent yields on the right ordinate are as a percent of the maximum glucose, xylose, or total glucose plus xylose possible.

yields of glucose in pretreatment (Stage 1) were less than 10% for both microwave and sand bath heating, yields of glucose from enzymatic hydrolysis (Stage 2) were much higher for solids from microwave heating than those from the sand bath, especially at shorter times of pretreatment. For example, the 72 h Stage 2 glucose percent yield for 5 min microwave pretreated corn stover was 65%, significantly higher than the 18% yield from corn stover pretreated for the same time in the sand bath. Combined Stage 1 + 2 glucose percent yields increased from 71% to 93% as the microwave heating pretreatment time was increased from 5 to 40 min, while the combined Stage 1 + 2 glucose percent yields for sand bath heating of corn stover increased from ~25% to 74%. Thus, microwave heating produced higher combined Stage 1 + 2 glucose percent yields than sand bath heating for hydrothermal pretreatment of corn stover at identical conditions (i.e., temperature, pretreatment time).

Fig. 1 shows that for sand bath heating, Stage 1 xylose percent yields continued to increase with pretreatment time until 40 min when the maximum xylose percent yield of 66% was reached. However, for microwave heating, Stage 1 xylose percent yields not only peaked at a slightly higher value of 68% but also much sooner at only 20 min. Xylose percent yields then declined to 47% at 40 min although xylan removal was as high as 91% (Table 3). The drop in xylose yield with longer pretreatment was due to greater xylose degradation by microwave heating at longer times. Thus, not surprisingly, Stage 2 xylose percent yields for microwave heating were generally less than those for sand bath heating because most of xylan had already been removed during microwave-heated pretreatment (Stage 1). As a result, the maximum combined Stage 1 + 2 xylose percent yield from sand bath heating was 96%, 12% higher than that from microwave heating. Interestingly, even though microwave heating showed more rapid xylan removal in Stage 1, combined Stage 1 + 2 xylose yields peaked at 20 min pretreatment time for both heating modes. Thus, microwave heating resulted in lower combined Stage 1 + 2 xylose percent yields even though Stage 1 yields from microwave heating were slightly higher.

In general, microwave heating resulted in higher combined Stage 1 + 2 glucose plus xylose percent yields for hydrothermal pretreatment than sand bath heating over pretreatment times

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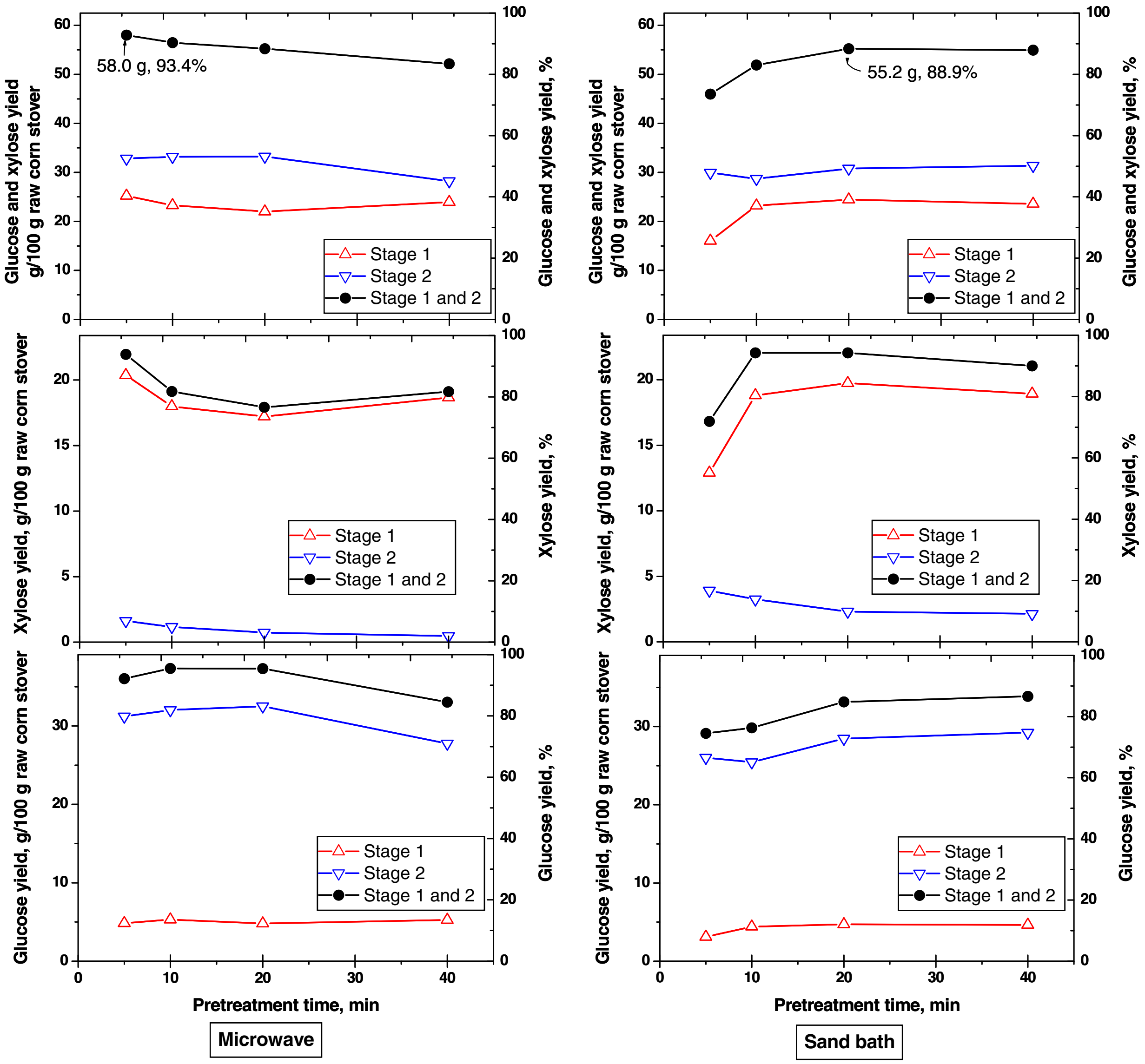

Fig. 2. Glucose, xylose, and total glucose plus xylose yields from pretreatment of corn stover 1 wt.% sulfuric acid at 140 °C and subsequent enzymatic hydrolysis of the resulting solids. Stage 1 refers to pretreatment, and Stage 2 refers to enzymatic hydrolysis. Mass sugars yields are reported on the left ordinate on the basis of 100g of corn stover being processed, dry basis, and percent yields on the right ordinate are as a percent of the maximum glucose, xylose, or total glucose plus xylose possible.

from 5 to 20 min. As shown in Fig. 1, as much as 50.3 g (81% of theoretical yield) of glucose and xylose were recovered from 100 g of raw corn stover for hydrothermal pretreatment at 180 °C for 20 min using sand bath heating followed by enzymatic hydrolysis. A slightly higher combined sugar recovery of 54.1 g/100 g raw corn stover or 87% of the combined Stages 1 + 2 percent sugar yield was achieved with microwave heating for 20 min at 180 °C. As the pretreatment time was extended to 40 min, increases in glucose yield compensated for losses in xylose yield for microwave heating, with the result that pretreatment with microwave heating gave a combined Stages 1 + 2 percent sugar yield of about 80%, comparable to that from pretreatment by sand bath heating.

3.4. Sugar yields from dilute acid pretreatment followed by enzymatic hydrolysis

Fig. 2 shows the same information on sugar release from Stage 1 and Stage 2 as Fig. 1, but now dilute acid pretreatment at 140 °C with sand bath and microwave heating. MW heating produced a slightly higher maximum Stage 1 glucose percent yield of about 15% compared to about 10% for SB heating. Peak Stage 2 glucose yields were also realized in less time for microwave than for sand bath heating. As shown in Fig. 2, a Stage 2 glucose percent yield of 81% was achieved after 10 min of microwave heating, about 15% higher than from sand bath heating for the same pretreatment time. Pretreatment by microwave heating for 10 min resulted in a maximum combined Stage 1 + 2 glucose percent yield of ~96%, while the maximum glucose percent yield of 75% from sand bath heating was reached in 40 min based on original glucan content in the raw corn stover. Thus, microwave heating produced a higher combined Stage 1 + 2 glucose yield at a shorter pretreatment time than sand bath heating for dilute acid pretreatment.

Stage 1 xylose percent yields peaked at 88% after only 5 min of dilute acid pretreatment with microwave heating and then gradually decreased to less than 80% as pretreatment continued. In contrast, only 53% Stage 1 xylose percent yield was achieved for 5 min of dilute acid pretreatment with sand bath heating. The peak Stage 1 xylose percent yield of 83% occurred at 20 min pretreatment time with sand bath heating and then decreased to 81% as the pretreatment time was increased to 40 min. The results in Table 3 suggest

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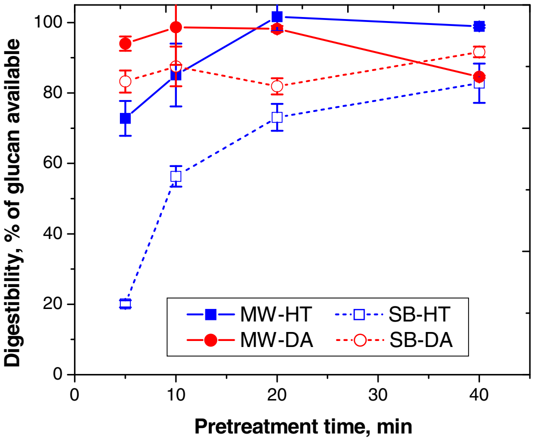

Fig. 3. Digestibility of solids resulting from hydrothermal (HT) and 1 wt.% sulfuric acid (DA) pretreatment of corn stover with microwave (MW) and sand bath (SB) heating expressed in g glucose released from the solids/maximum amount of glucose available. The enzyme loading was 50 mg of protein/(g glucan plus xylan) in the original biomass (equivalent to about 30 FPU/g glucan in pretreated solids).

that microwave heating sped up xylan solubilization during pretreatment, with the result that less xylan remained in the MW pretreated solids compared with those from sand bath heating. Stage 2 xylose percent yields were low for both microwave heating (<7%) and sand bath heating (<18%), mainly due to nearly complete xylan solubilization during pretreatment, especially for pretreatments longer than 20 min. The combined Stage 1 + 2 xylose percent yield for 5 min of microwave pretreatment with dilute acid was comparable to that from sand bath heating applied to dilute acid pretreatment for 10 min.

Microwave heating gave the highest combined Stage 1 + 2 glucose plus xylose yields, with 20 min being required to reach peak yields for hydrothermal pretreatment, while dilute acid only needed 5 min to maximize the total sugar yield. At pretreatment conditions of 140 °C with 1 wt.% sulfuric acid for 5 min, microwave heating achieved the highest combined Stage 1 + 2 glucose percent yields of about 93% and combined Stage 1 + 2 xylose percent yields of 94%, respectively, providing a combined Stage 1 + 2 total sugar yield of over 93%. However, although applying microwave heating to dilute acid pretreatment resulted in peak sugar yields in much shorter reaction times than sand bath heating, the combined Stage 1 + 2 total sugar percent yield then decreased with increasing pretreatment time, dropping to ~82% for a pretreatment time of 40 min. On the other hand, even though sand bath heating required pretreatment for at least 20 min to maximize combined Stage 1 + 2 total sugar yields at almost 89%, extending the pretreatment time to 40 min had little effect on the combined Stages 1 + 2 percent sugar yield.

3.5. Enzymatic digestibility of solids from microwave vs. sand bath heating

Fig. 3 demonstrates that microwave heating significantly enhanced enzymatic release of glucose (glucan digestion) from the glucan left in the solids following either hydrothermal or dilute acid pretreatment of corn stover, especially at shorter pretreatment times, with essentially complete digestion of glucan possible at the enzyme loading applied for microwave heating of either pretreatment. For hydrothermal pretreatment, microwave heating improved glucan digestion by 19–52% compared with sand bath heating over the range of times covered. Furthermore, greater than 80% digestion was achieved for microwave heating at 180 °C at a much shorter time of 10 min, compared to 40 min to reach similar digestibility for sand bath heating of hydrothermal pretreatment. The impact of microwaves was less dramatic for dilute acid pretreatment, with microwave heating increasing glucan digestibility by only 6–11% compared to sand bath heating. Yet, despite these significant effects on glucose release from the glucan in the pretreated solids, there was no clear influence on xylose release by enzymatic hydrolysis of pretreated solids (data not shown), mostly due to nearly complete xylan removal by microwave pretreatment prior to enzymatic hydrolysis, as shown in Table 3.

3.6. Acetyl group removal for microwave vs. sand bath

Fig. 4a shows the acetyl content left in microwave and sand bath heated solids expressed as a percent of the amount in the corn stover originally over time for both hydrothermal and dilute acid pretreatments. For hydrothermal pretreatment, microwave heating removed about 10–30% more acetyl content than sand bath heating, and produced similar results for dilute acid pretreatment. For example, microwave heating removed about 90% of the acetyl content following 20 min of dilute acid pretreatment at 140 °C, while sand bath heating released only 60% of the acetyl content in the same time period.

The relationship between digestibility of glucan left in the pretreated solids and acetyl removal by pretreatment shown in Fig. 4b reveals that glucan digestibility generally increased with greater acetyl content removal. For example, results from this study

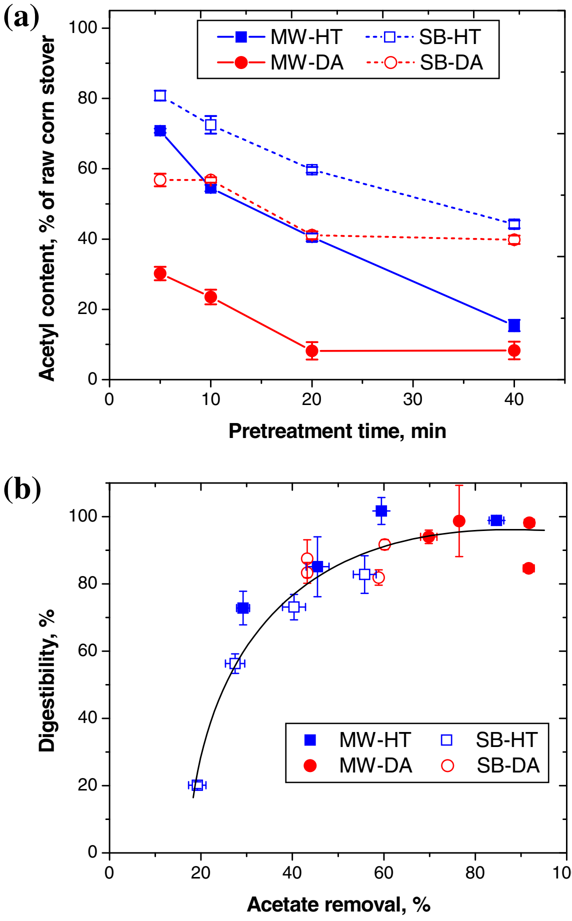

Fig. 4. (a) Acetyl content remaining in solids following hydrothermal (HT) and dilute acid (DA) pretreatment of corn stover with microwave (MW) and sand bath (SB) heating at various times; (b) Correlation between glucan digestibility and acetyl removal from solids following pretreatment with microwave (MW) and sand bath (SB) heating.

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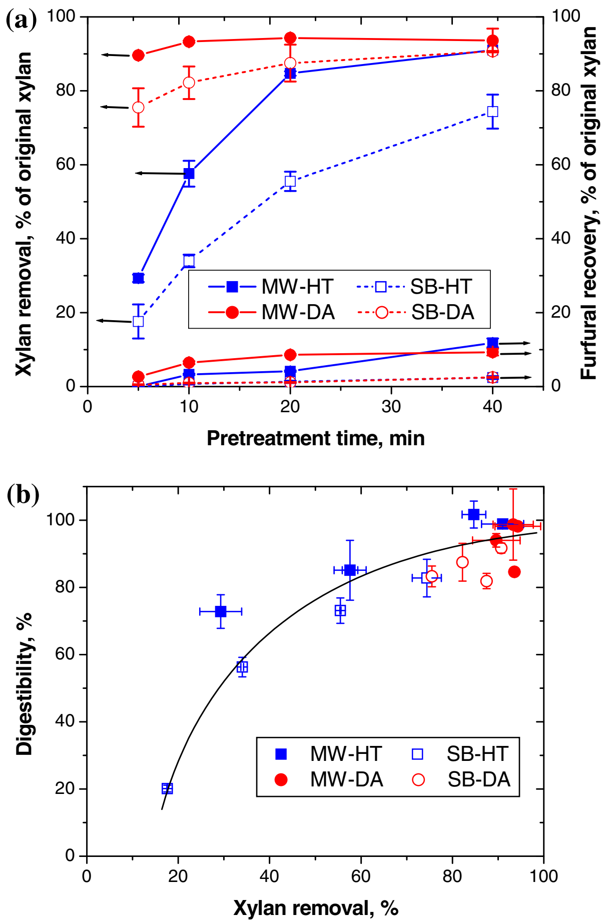

Fig. 5. (a) Xylan removal and furfural recovery resulting from hydrothermal (HT) and dilute acid (DA) pretreatment of corn stover with microwave (MW) and sand bath (SB) heating; (b) Correlation between cellulose digestibility and xylan removal for microwave (MW) and sand bath (SB) heating.

showed that at the point of removal of 30–90% of the acetyl groups in microwave heating, glucan digestibility was 70–99%, while when 20–60% of the acetyl content was removed by sand bath heating, the glucan digestibility was only 20–90% in enzymatic hydrolysis.

3.7. Xylan removal and degradation for microwave and sand bath heating

Fig. 5a compares xylan removal for microwave heating to that for sand bath heating for both hydrothermal and dilute acid pretreatments. Microwave heated hydrothermal pretreatment at 180 °C resulted in 30–90% xylan removal, about 10–30% higher than for sand bath heating. Similarly, applying microwave heating to dilute acid pretreatment removed more than 90% of the xylan from the solids after only 5–10 min of pretreatment. However, sand bath heating required a pretreatment time of about 40 min to achieve the same extent of xylan removal.

Fig. 5b shows that glucan digestion during enzymatic hydrolysis increased with xylan removal. Consistent with this trend, because microwave heating removed xylan faster than sand bath heating, the digestibility of the solids from microwave heating was greater than that from sand bath heating for hydrothermal pretreatment at the same pretreatment times. However, it is interesting that the digestibility of the solids was somewhat higher for microwave heating than for sand bath heating at similar extents of xylan removal until about 90% of the xylan had been removed, at which point they become similar.

Fig. 5a also shows that more furfural was formed for microwave heating than for sand bath heating at the same conditions, likely because more xylan was in solution to be degraded to furfural. For dilute acid pretreatment, as expected, little furfural formation was detected with sand bath heating due to the lower reaction temperature of 140 °C. However, microwave heating produced substantial amounts of furfural, especially for longer pretreatment times. For example, as shown in Fig. 5a, 10% of the xylan was degraded to furfural after 40 min of microwave heated dilute acid pretreatment. Hydrothermal pretreatment at the higher temperature of 180 °C produced similar results. In this case, microwave heating degraded up to 11% of the original xylan, but less than 2% of the xylan was lost to furfural and other degradation products for sand bath heating for up to 40 min during hydrothermal pretreatments.

3.8. Solid state CP/MAS NMR characterization

The CP/MAS ^{13}C NMR spectra of raw control and pretreated corn stover are observed to dominate by the set of resonances in the 58–110 ppm region corresponding to cellulose signals (see Supporting material). The signal for the anomeric carbon (C-1) of cellulose appeared as a single peak centered at 105 ppm; the C-2, C-3, and C-5 carbons of cellulose overlapped at the peaks around 72 and 75 ppm; and the C-6 carbon of cellulose resonated between 58 and 64 ppm (Pu et al., 2006; Samuel et al., 2010). The C-4 carbon arising from crystalline and amorphous cellulose was centered at peaks of ~89 and 84 ppm, respectively. Pretreatment significantly changed the relative signal intensity of peaks at 89 and 84 ppm. Compared to raw control corn stover, the peak at ~84 ppm showed a reduction in signal intensity after microwave-heating and sand bath hydrothermal pretreatment, which was largely attributed to the loss of amorphous cellulose and other composites such as hemicellulose. For both microwave and sand bath heating pretreatments, signal intensities for the amorphous regions dropped significantly as pretreatment time increased. Compared to sand bath hydrothermal pretreatment, microwave pretreatment at the same pretreatment time led to a lower signal intensity for the amorphous regions, suggesting that microwave heating degraded or otherwise altered the amorphous regions of corn stover more than sand bath heating did.

4. Discussion

This study provides the first comparison of microwave and sand bath heating for hydrothermal and dilute acid pretreatment of cellulosic biomass, in this case, corn stover, in terms of sugar release from pretreatment and subsequent enzymatic hydrolysis. Hydrothermal and dilute acid pretreatments are typically performed at temperatures between about 140 and 220 °C, and cellulosic biomass solids limits convective heat transfer by fluid motion. Consequently, heating of the contents is typically by conduction through the water and the biomass solids, resulting in temperature gradients (Jacobsen and Wyman, 2001; Stuhler and Wyman, 2003; Viamajala et al., 2010). Because the reaction kinetics have been shown to follow Arrhenius behavior, the rates change substantially with temperature, and sugar yields from cellulose and hemicellulose hydrolysis are very sensitive to the temperature history (Jacobsen and Wyman, 2001). To minimize such effects at the laboratory scale, small diameter tube reactors should be used to study hemicellulose hydrolysis kinetics for heating in fluidized sand baths, oil baths, steam, or other approaches with external heating, but even then, the extent of hydrolysis can vary across

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the tube radius due to temperature gradients for conduction-based heating (Jacobsen and Wyman, 2001; Stuhler and Wyman, 2003). Microwave radiation, on the other hand, heats objects directly by an electromagnetic field, thus avoiding temperature gradients across the tube radius. On the other hand, microwaves can introduce non-thermal effects and possibly hot spots that could affect the performance for cellulosic biomass pretreatment (Hu and Wen, 2008).

In this study, application of microwave heating to hydrothermal pretreatment resulted in higher sugar yields in less time than sand bath heating, with higher xylan solubilization realized at shorter pretreatment times of only 5–10 min. However, microwave heating also led to greater xylan/xylose degradation, as indicated by the higher furfural content, especially at the higher pretreatment severities applied. As a result, combined Stage 1 + 2 xylose yields for longer pretreatment times of 20–40 min were lower for microwave heating. The patterns were similar for dilute acid pretreatment in that high xylose yields from microwave heated pretreatment were achieved at much shorter pretreatment times than needed to realize comparable yields for sand baths.

Besides xylan removal, microwave heating led to higher acetyl removal and slightly higher lignin removal than sand bath heating. Acetyl removal and lignin removal have been considered to be associated with xylan removal and were both reported to enhance enzymatic digestion due to enhanced cellulose/xylan accessibility and enzyme effectiveness (Kim and Holtzapple, 2006). For both hydrothermal and dilute acid pretreatments, microwave heating led to significantly higher glucan digestibility in pretreated solids by enzymes compared with sand bath heating, with the effect more prominent for hydrothermal pretreatment. The enhanced glucan digestibility can be related to the combined effects of xylan, acetyl, and lignin removal, each of which may increase the surface area of lignocellulosic material (Mansfield et al., 1999). Lignin modification could also make polysaccharides more susceptible to enzymatic hydrolysis as suggested by many other studies (Jorgensen et al., 2007; Yang and Wyman, 2004).

From the CP/MAS NMR analysis, microwave heating led to greater removal of the amorphous fraction of lignocellulosic biomass. Yet, removal of lignin and xylan during pretreatment could improve enzyme access to cellulose and reduce non-productive binding of enzyme despite the increase in crystallinity index. As suggested by previous studies, increased accessibility plays a significant role in enhancing cellulose digestion by enzymes (Kumar et al., 2009; Mosier et al., 2005). Therefore, comprehensive effects from lignin, xylan removal, and alteration of cellulose structural features during microwave heating may contribute to overall enhanced enzymatic digestion compared to sand bath heating.

Through side by side comparisons of microwave heating and sand bath heating, it is evident that microwave heating resulted in faster pretreatment and comparable overall sugar yields with a higher proportion of glucose in the sugars released compared to sand bath heating. Thus, microwave heating enhanced both hydrothermal and dilute acid pretreatment of corn stover and subsequent enzymatic hydrolysis of the pretreated solids. Cellulosic biomass is a highly heterogeneous complex of polymers, primarily lignin, cellulose, and hemicelluloses, coupling with the complication introduced by the catalyst used during pretreatment, i.e., carboxylic acid released from water by autohydrolysis of hemicelluloses and added sulfuric acid. In addition, the high water content of cellulosic biomass is susceptible to microwave heating. Considering all these factors, application of microwaves to lignocellulose may selectively heat the more polar part and create “hot spots” within heterogeneous materials (Lewis et al., 1992). This unique heating feature could possibly result in an “explosion” effect among the particles and improve disruption of the recalcitrant structures of lignocellulose (Hu and Wen, 2008). In addition, the electromagnetic field of microwaves might create non-thermal effects that improve release of hemicellulose sugars, although no direct evidence has been reported previously (de la Hoz et al., 2005).

5. Conclusions

Times for heat up and cool down were similar for microwave and sand bath heating of tubular batch pretreatment reactors. However, although overall sugar yields from both were comparable, the times to maximum yields were much different, with microwave heated pretreatment giving faster xylan, lignin, and acetyl removal, and faster xylose release for microwave heating resulted in more degradation. Microwave pretreatment demonstrated more effective breakdown of amorphous regions of corn stover than sand bath heating and resulted in higher glucose yields from enzymatic hydrolysis of pretreated corn stover. Similar trends were found for both hydrothermal and dilute acid pretreatment.

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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.2011.03.027.

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