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Improvement of enzymatic saccharification of *Populus* and switchgrass by combined pretreatment with steam and wet disk milling

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ABSTRACT

To reduce the recalcitrance of lignocellulosic biomass for subsequent biological processing, we pretreated energy crop feedstocks with mild steam treatment (ST; 130 and 150 °C for 60 min) and wet disk milling (WDM). We tested two phylogenetically different, but typical energy crop feedstocks: *Populus trichocarpa* and switchgrass (*Panicum virgatum*). WDM after ST facilitated the fibrillation of both types of biomass, resulting in an increase of specific surface area, improved enzymatic saccharification yield, and decrease in cellulose crystallinity. After steam treatment at 150 °C followed by 17 cycles of WDM, enzymatic hydrolysis resulted in almost complete glucan to glucose conversion in both feedstocks.

1. Introduction

Lignocellulosic feedstocks, such as forest residues, wood process wastes, and the organic fraction of municipal solid wastes, have attracted attention as a sustainable resource for the production of various chemical products, including fuels, fine chemicals, and value-added materials. With proper processing, these lignocellulosic wastes and residues can be used to produce renewable chemical products without substantially increasing arable land requirements or impacting food and fiber crop production [1]. However, supplies of these lignocellulosic feedstocks are limited in many regions. Therefore, additional sources of herbaceous grasses or short rotation forest crops are required to expand production of biomass-based fuels and chemicals. Dedicated energy crop feedstocks, such as switchgrass, *Miscanthus*, sorghum, *Populus*, *Eucalyptus*, and willow, are promising biomass materials because they have a fast growth rate, flexible harvest times, are mostly perennials, can grow on marginal or degraded land, and have high available carbon content. In addition, their geographic distribution and production have been studied and breeding research that includes genetic modification has been conducted in order to increase the yields of these plants [2–5]. *Populus trichocarpa* and switchgrass (*Panicum virgatum*) have been especially widely studied as candidate energy crops that are woody and herbaceous, respectively [6–8].

Lignocellulose contains chemically and structurally complex polymeric macromolecules composed of three main structural components, i.e., cellulose, hemicelluloses, and lignin [9]. Its complex structure makes lignocellulose inherently difficult to break down. This is referred to as biomass recalcitrance. A large number of pretreatment processes have been developed to overcome biomass recalcitrance and to promote the effective enzymatic conversion of carbohydrate polymers into monomeric sugars [10,11]. *Populus* and switchgrass have been subjected to various

Abbreviations: steam treatment, ST; wet disk milling, WDM; hot-compressed water treatment, HCWT; high-performance liquid chromatography, HPLC; specific surface area, SSA; crystallinity index, CrI; scanning electron microscope, SEM.

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pretreatment approaches. Pretreatments for *Populus* and switchgrass have included thermochemical pretreatments (e.g., steam explosion and hot-compressed water treatment (HCWT)) [12–14], mechanical size reduction by grinding (e.g., hammer milling, ball milling, and twin-screw extrusion) [15–17], and chemical pretreatments (dilute acid, alkaline, organic solvents, and ionic liquid) [18–21].

Recently, our research group described a pretreatment method combining HCWT with wet disk milling (WDM) [22]. WDM is a mechanical size reduction process that fibrillates the plant cell wall to nanoscale by the application of shear force and pressure under wet conditions [23]. The fibrillation process increases enzyme accessibility and enzymatic degradability, but the process uses a large amount of energy. HCWT facilitates mechanical refinement of WDM and reduces the amount of energy needed for this process by partially dissolving hemicelluloses and lignin [22]. This pretreatment is regarded as environmentally friendly because no chemicals are used. Although high-temperature HCWT can promote the weakening of lignocellulosic biomass, the severe conditions of HCWT also cause excessive biomass degradation and generate by-products that can inhibit saccharification and fermentation. HCWT is usually conducted at temperatures greater than 150 °C and various pressures, because water exhibits exciting physical and chemical properties at those temperatures, depending on the pressure [24–26]. Yu et al. [27] reported effective degradation of hemicellulose and high sugar production yields from rice straw pretreated at 180 °C, but also reported that the formation of fermentation inhibitors such as acetic acid and furfural, significantly increased with pretreatment at temperatures greater than 200 °C. Furthermore, re-localization of lignin on cellulose surfaces was reportedly caused by high-temperature thermochemical pretreatment [28]. Re-localized lignin reduces enzyme accessibility.

In this study, we show results from steam treatment (ST) at temperatures up to 150 °C combined with WDM to increase fibrillation of *Populus* and switchgrass feedstocks. Although ST is conducted at low temperatures, ST may promote enzymatic degradability when combined with WDM [29]. The goal was to improve enzymatic saccharification yield while avoiding the production of fermentation inhibitors by using a thermochemical pretreatment that is milder than HCWT.

2. Materials and methods

2.1. Materials

P. trichocarpa (black cottonwood) was obtained from a tree growing on the property of Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. *Panicum virgatum* (switchgrass) was provided by the Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA. *Populus* and switchgrass were milled to less than 3 mm using cutter mill (MKCM-3, Masuko Sangyo Co., Ltd., Saitama, Japan) under dry conditions and were stored at room temperature.

Three commercial enzymes were mixed and used for enzymatic saccharification. Acremonium cellulase (Meiji Seika Co., Tokyo, Japan) derived from *Talaromyces cellulolyticus* (formerly known as *Acremonium cellulolyticus* [30]) was used as a source of cellulase. Optimash BG (β-glucanase/xylanase, Genencor International, Palo Alto, CA, USA) and Novozyme 188 (β-glucosidase, Novozymes, Franklinton, NC, USA) were used as supplementary enzymes. Other chemicals of the highest grade were obtained from commercial sources.

2.2. Chemical composition analysis

The chemical compositions of *Populus* and switchgrass were determined before and after ST based on the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (NREL) with some modifications [31]. Before chemical composition analysis, all samples were freeze-dried and then vacuum-dried at 40 °C for 18 h. The dried samples (50 mg) were hydrolyzed by shaking with 72 wt% sulfuric acid (600 μL) for 90 min at 30 °C. After diluting the acid to a final concentration of 4 wt% by adding 16.8 mL Milli-Q water, diluted samples were autoclaved at 121 °C for 1 h. The autoclaved samples were separated into supernatant and residue by filtration using PTFE-membrane filters. The supernatant was neutralized with saturated barium hydroxide, and the monomeric sugars in the supernatant were analyzed using a high-performance liquid chromatography (HPLC) system consisting of an LC-2000 Plus (Jasco Co., Ltd., Tokyo, Japan) equipped with an Aminex HPX-87P column (Bio-Rad Labs., Hercules, CA, USA). HPLC analysis was conducted at 60 °C with a flow rate of 0.25 mL Milli-Q water/min. The acid-insoluble lignin (Klason lignin) content was determined by weighting the vacuum-dried residue.

2.3. Steam treatment (ST)

Dried biomass samples were soaked in water to adjust the concentration of samples in water to 10 wt% and left overnight at room temperature. The soaked samples were milled using cutter mill (MKCM-3, Masuko Sangyo Co., Ltd., Saitama, Japan) under wet conditions. ST was then performed in a stainless steel-bucket at 130 or 150 °C for 1 h using an autoclave (MCS-3032, ALP Co. Ltd., Tokyo, Japan), as previously described [29]. Fig. 1 shows representative heating and cooling temperature profiles for ST at 130 and 150 °C. After ST, a portion of each sample was removed, filtered, and washed with Milli-Q water. The water-soluble fractions were used for analysis of monomeric sugars without any additional treatment. Total sugar content was determined by total hydrolysis with 4 wt% sulfuric acid using the method following the LAP of the NREL [32]. The concentration of each sugar was determined by using HPLC as described in 2.2.

2.4. Wet disk milling (WDM)

Hydrothermally treated samples were diluted to 3 wt% without removing water-soluble fractions. Samples were then subjected to WDM using a disk mill (Supermasscolloider MKCA6-2, Masuko

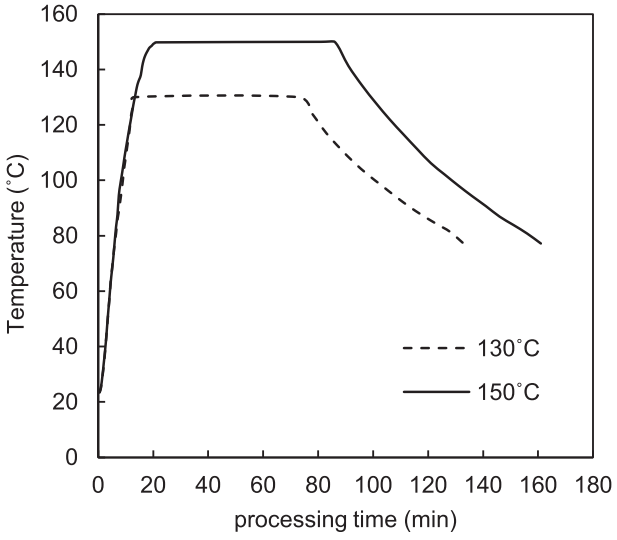

Fig. 1. Temperature profiles of steam treatment (ST) at 130 °C (dashed line) and 150 °C (solid line).

Table 1
Chemical composition of untreated and steam-treated *Populus* and switchgrass.

Sample	Temperature condition (°C)	Glucose (%)	Xylose (%)	Galactose (%)	Arabinose (%)	Mannose (%)	Acid-insoluble lignin (%)
Populus	Untreated	55.93 (1.38)	15.25 (0.19)	1.08 (0.38)	1.10 (0.26)	3.45 (0.47)	24.14 (0.42)
	130	50.35 (0.29)	14.50 (1.49)	0.95 (0.26)	0.66 (0.33)	2.58 (0.54)	23.95 (0.27)
	150	50.74 (2.07)	14.52 (0.98)	0.92 (0.25)	0.74 (0.21)	2.75 (0.38)	23.65 (0.34)
Switchgrass	Untreated	40.18 (0.84)	26.57 (0.83)	1.36 (0.07)	3.40 (0.13)	–	23.36 (0.32)
	130	39.82 (1.60)	26.88 (1.07)	1.25 (0.24)	3.00 (0.01)	–	22.05 (0.17)
	150	41.83 (1.99)	27.61 (1.10)	1.20 (0.27)	3.22 (0.37)	–	21.90 (0.09)

The data are mean values of duplicate analyses (standard errors in parentheses).

Sangyo Co., Ltd., Saitama, Japan) following the method described in previous reports [22,33]. Each sample underwent a total of 17 milling cycles with a clearance of 20–40 μm and rotation speed at 1800 rpm. Each milling cycle was timed, and the milling time per unit material weight was calculated.

2.5. Enzymatic saccharification

Enzymatic hydrolysis was performed using an enzyme cocktail containing Acremonium cellulase, Optimash BG, and Novozyme 188. The concentrations of the pretreated samples were adjusted to 3 mg/mL, and enzymes were added at the following concentrations: 10 FPU/g biomass sample of Acremonium cellulase, and to improve the efficiency of enzymatic hydrolysis, 0.02 mL/g biomass sample of Optimash BG β-glucanase/xylanase, and 5 IU/g biomass sample of Novozyme 188 β-glucosidase. The final reaction solution volume was 2.5 mL and solutions were prepared in 5-mL tubes with 50 mM acetate buffer (pH 5.0). Reactions were carried out at 50 °C for 72 h with agitation using rotary shaker set at 220 rpm. After 72-h of saccharification, the enzymes were heat-inactivated at 95 °C for 10 min. The inactivated solutions were centrifuged for 5 min at 10,000 rpm, and the collected supernatants were subjected to sugar analyses as described in 2.2.

2.6. Morphology, specific surface area (SSA), and crystallinity index (CrI)

Samples used for characterization of morphology and analyses of SSA and CrI were washed with *tert*-butyl alcohol several time until the water was thoroughly replaced by *tert*-butyl alcohol. Samples were then freeze-dried to preserve the original structure before analysis. Morphological characteristics were observed using an S-4800 scanning electron microscope (SEM; Hitachi Co., Tokyo, Japan). SSAs were determined from a Brunauer–Emmett–Teller plot of the nitrogen adsorption–desorption isotherm using a BELSORP-Max (BEL Japan Inc., Osaka, Japan) [34]. X-ray diffraction experiments were carried out to determine CrI using a RINT-TTR III diffractometer (Rigaku, Tokyo, Japan), and the CrIs were calculated by the Segal method [35].

3. Results

3.1. Combination of ST and WDM

The chemical compositions of untreated and steam-treated samples are summarized in Table 1. Mild-temperature ST had little effect on the chemical composition of *Populus* and switchgrass. ST especially decreased the amount of glucose in *Populus* but did not significantly affect other sugar components and acid-insoluble lignin in either biomass feedstocks.

Table 2 shows the concentration of monomeric (M) and total (T) sugars in the water-soluble fraction after ST. Total sugars were analyzed after acid hydrolysis and differences between total sugars and monomeric sugars indicate the presence of sugar oligomers not detected by HPLC analysis. ST at 150 °C liberated more sugars than ST at 130 °C. Xylose was the main sugar in the water-soluble fraction of both feedstocks. The concentration of total sugars was higher in switchgrass than in *Populus*, indicating that the hemicelluloses in switchgrass are more easily degraded by ST than those in *Populus*. As shown in Table 1, the constituents of the hemicellulose in switchgrass differ from those in *Populus*, and a proportion of the monosaccharides forming hemicellulose are higher in switchgrass than in *Populus*. In general, acetylated glucuronoxylan is a major hemicellulose component in hardwoods, including *Populus* [36], whereas in herbaceous monocots like switchgrass, glucurono-arabinoxylan is a major hemicellulose component [37]. The differences between the amounts sugars in the water-soluble fraction from each feedstock may be due to differences in the amount and molecular structure of the hemicellulose contained in the plants.

Some degradation products from hydrothermal treatment are known as fermentation inhibitors such as acetic acid, furfural, 5-hydroxymethylfrufural, formic acid, 2-furoic acid, and levulinic acid [25]. All of these fermentation inhibitors, except for 2-furoic

Table 2
Concentration of monomeric (M) and total (T) sugars in the supernatant after steam treatment (ST) of *Populus* and switchgrass.

Sample	Temperature condition (°C)	Concentration (mg/g of biomass)												
		Cellobiose		Glucose		Xylose		Galactose		Arabinose		Mannose		Total[a]
		(M)	(T)	(M)	(T)	(M)	(T)	(M)	(T)	(M)	(T)	(M)	(T)	(T)
Populus	130	0.17 (0.01)	–	0.15 (0.01)	1.10 (0.11)	0.08 (0.00)	1.59 (0.11)	–	0.74 (0.05)	0.14 (0.01)	1.07 (0.10)	–	0.91 (0.18)	5.41
	150	0.45 (0.01)	–	0.31 (0.01)	1.72 (0.23)	0.52 (0.05)	18.61 (0.34)	0.25 (0.02)	3.50 (0.24)	0.75 (0.01)	2.24 (0.12)	–	1.74 (0.07)	27.81
Switchgrass	130	1.12 (0.08)	–	4.30 (0.10)	12.03 (0.47)	0.28 (0.01)	3.68 (0.10)	–	1.99 (0.25)	1.72 (0.06)	3.48 (0.39)	–	–	21.18
	150	1.34 (0.16)	–	5.52 (0.07)	19.77 (0.81)	1.11 (0.08)	27.64 (1.44)	0.73 (0.06)	6.26 (0.73)	5.62 (0.10)	10.81 (0.60)	–	–	64.48

Monomeric (M) sugars in the liquid fractions were analyzed by HPLC without additional treatment and total (T) sugars in the liquid fractions were analyzed using a sulfuric acid hydrolysis method followed by HPLC determination.
The data are mean values of duplicate analyses (standard errors in parentheses).
[a] The total value is the sum of the concentration of each total sugar.

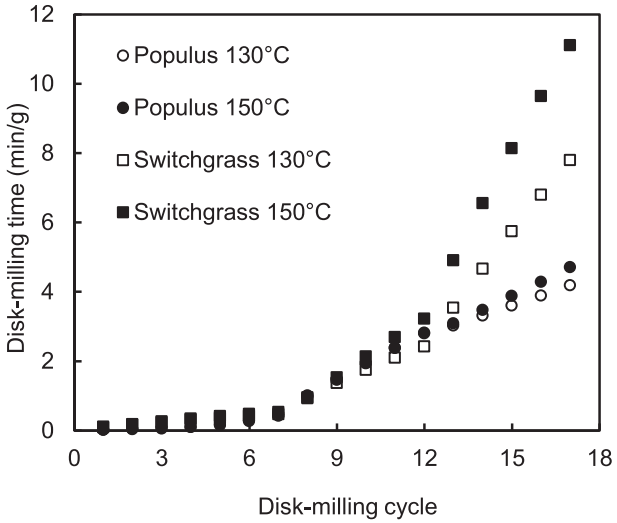

Fig. 2. Relationship between disk-milling cycle and disk-milling time. Circles and squares indicate *Populus* and switchgrass, respectively. White and black indicate ST temperatures of 130 and 150 °C, respectively.

acid and levulinic acid, were detected in every water-soluble fraction after ST. However, all of these inhibitors were present only in very small amounts of up to 40 mg/L (data not shown). At such low concentrations, these degradation products would have an insignificant effect on the growth of various fermentative microorganisms [38]. In addition, the glucose production yields of the pretreated product with and without the water-soluble fraction were similar. These results indicate that the mild-temperature ST used in this study can limit the production of inhibitors of saccharification and fermentation.

Fig. 2 shows the relationship between disk-milling cycle and time. Generally, well-fibrillated samples have high viscosity and require a long disk-milling time. The disk-milling time for each cycle was similar between *Populus* and switchgrass until 12 cycles, but became to differ between *Populus* and switchgrass over 13 cycles. At each cycle, both *Populus* and switchgrass samples treated at 150 °C took slightly longer to mill than those treated at 130 °C. Meanwhile, the dependence of disk-milling time on temperature was greater for switchgrass than for *Populus*. There was more hemicellulose in switchgrass than in *Populus* (Table 1) and the hemicellulose of switchgrass was more susceptible to the effects of hydrothermal treatment than that of *Populus*. Susceptibility of hemicelluloses to ST temperature may affect the dependence of disk-milling time on temperature.

3.2. Morphology, SSA, and CrI

Fig. 3 shows SEM images of biomass samples after ST at different temperatures and subsequent WDM with different numbers of disk-milling cycles. Samples treated by only ST showed micron-scale fibers and some additional fuzzy fibers on the surfaces of the microscopic fibers (Fig. 3 a–d). Nanoscopic fibrous structures were observed after fibrillation by WDM (Fig. 3 e–h). As disk-milling time increased, nano-scale fibers became much finer, and area that included nanoscopic fibers increased (Fig. 3 i–l). These morphological changes were observed in all samples, regardless of

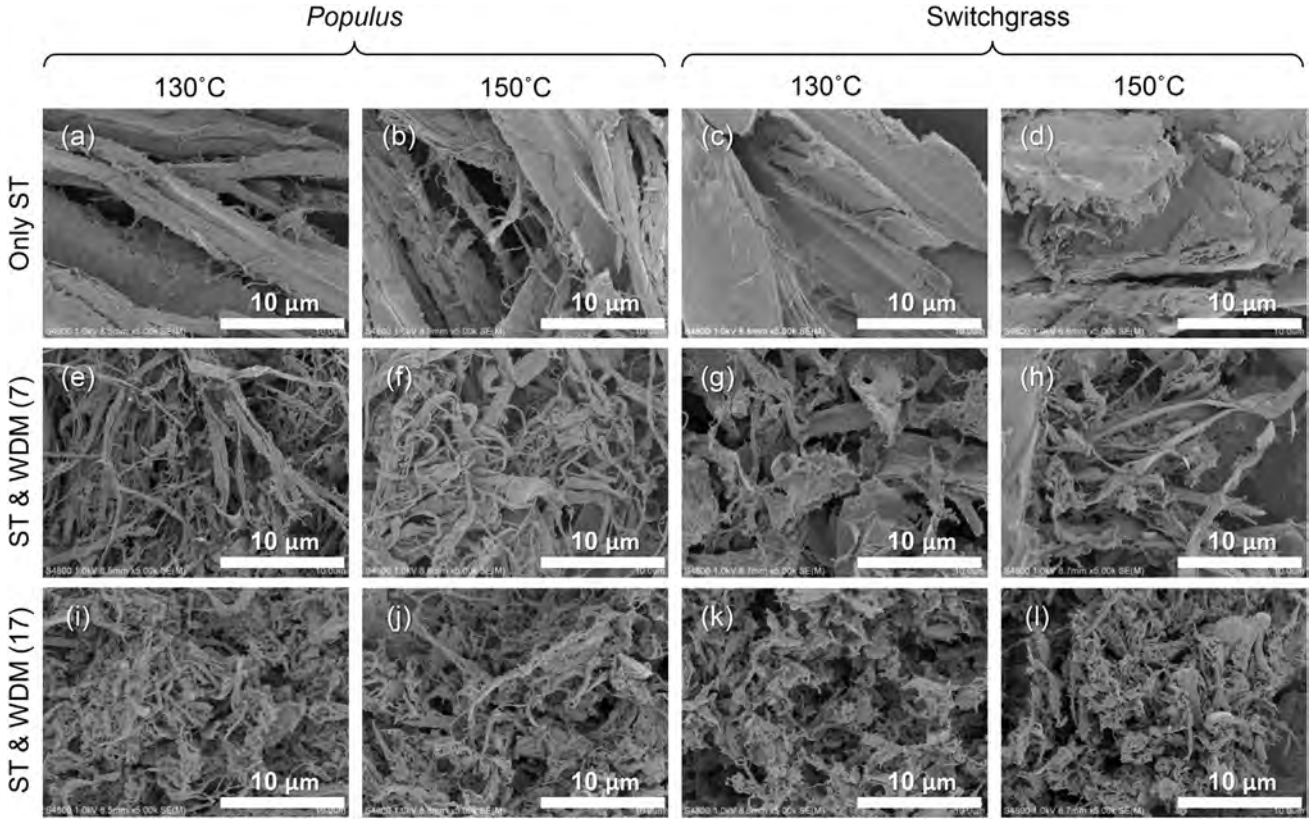

Fig. 3. SEM images of *Populus* and switchgrass treated by steam treatment (ST) combined with wet disk milling (WDM). (a–d) ST without WDM, (e–h) ST with 7 cycles of WDM, (i–l) ST with 17 cycles WDM. (a, e, and i) *Populus* steam-treated at 130 °C, (b, f, and j) *Populus* steam-treated at 150 °C, (c, g, and k) switchgrass steam-treated at 130 °C, (d, h, and l) switchgrass steam-treated at 150 °C.

plant species and temperature conditions. Therefore, the combination of ST and WDM has good potential to effectively promote fibrillation of various lignocellulosic biomass sources.

To quantitatively evaluate the morphologies of samples, we determined the SSA of pretreated samples. SSA results are summarized along with CrI results in Fig. 4. Although CrI values do not directly indicate morphologic characteristics, the effects of the pretreatment on the physicochemical properties of biomass can be evaluated using CrI [39]. The SSA values of both *Populus* and switchgrass increased with increasing disk-milling cycles, but at the same disk-milling cycle, there was little difference in SSA values between treatment temperatures (Fig. 4a, b). Increased SSA indicates progression of fibrillation and results in an increase in the proportion of cellulose that is exposed to enzymes. The SSA results were related to the results of SEM observation (Fig. 3). Fibrillation increased substantially with additional disk-milling cycles, but both treatment temperatures resulted in nearly the same morphology. The CrI values of both *Populus* and switchgrass gradually decreased with increasing disk-milling cycles in the same way (Fig. 4c, d). The CrI values indicated small differences between treatment temperatures at the same disk-milling cycle. In addition, the SSA and CrI values for *Populus* were consistently higher than those of switchgrass at the same disk-milling cycle (Fig. 4).

3.3. Enzymatic saccharification

Fig. 5 shows the relationship between disk-milling time and glucose production yield. The relationships between disk-milling time and glucose production yield were slightly different for *Populus* and switchgrass. *Populus* required less disk-milling time to achieve a high glucose production yield than switchgrass, whereas the glucose production yield of switchgrass gradually increased with disk-milling time. As shown in Fig. 2, the disk-milling time for each cycle became to differ between *Populus* and switchgrass over 13 cycles, and the disk-milling times at 17 cycles of switchgrass were more than twice longer than those of *Populus*. Therefore, the profile of the relationship between disk-milling time and glucose production yield differed between *Populus* and switchgrass. However, the glucose yields were obviously higher for both feedstocks when treated at 150 °C than when treated at 130 °C (Fig. 5).

The sugar production yields of 17-cycle disk-milled samples and untreated samples are shown in Fig. 6. For both *Populus* and switchgrass, production yields of xylose and glucose were greater than 90% after ST at 150 °C. In addition, another sugar was also detected in the reaction solution of each sample after enzymatic saccharification, and mannose and arabinose were detected in *Populus* and switchgrass, respectively. Each sugar was second most constituent sugar of *Populus* and switchgrass after xylose except for glucose (Table 1). The production yields of these sugars for *Populus* and switchgrass treated at 150 °C were 49.3% and 66.8%, respectively. All sugar production yields of 17-cycle disk-milled samples were significantly improved by ST at a higher temperature.

4. Discussion

Hydrothermal treatment is typically conducted at the temperatures greater than 150 °C, and the degradation of hemicellulose begins at around 180 °C [24,25]. Therefore, 130 and 150 °C are mild-temperature conditions for hydrothermal treatment. The glucose production yields of all samples treated by ST only at 130 and 150 °C after enzymatic saccharification were around 20% (Fig. 5). The glucose production yields of all steam-treated samples gradually

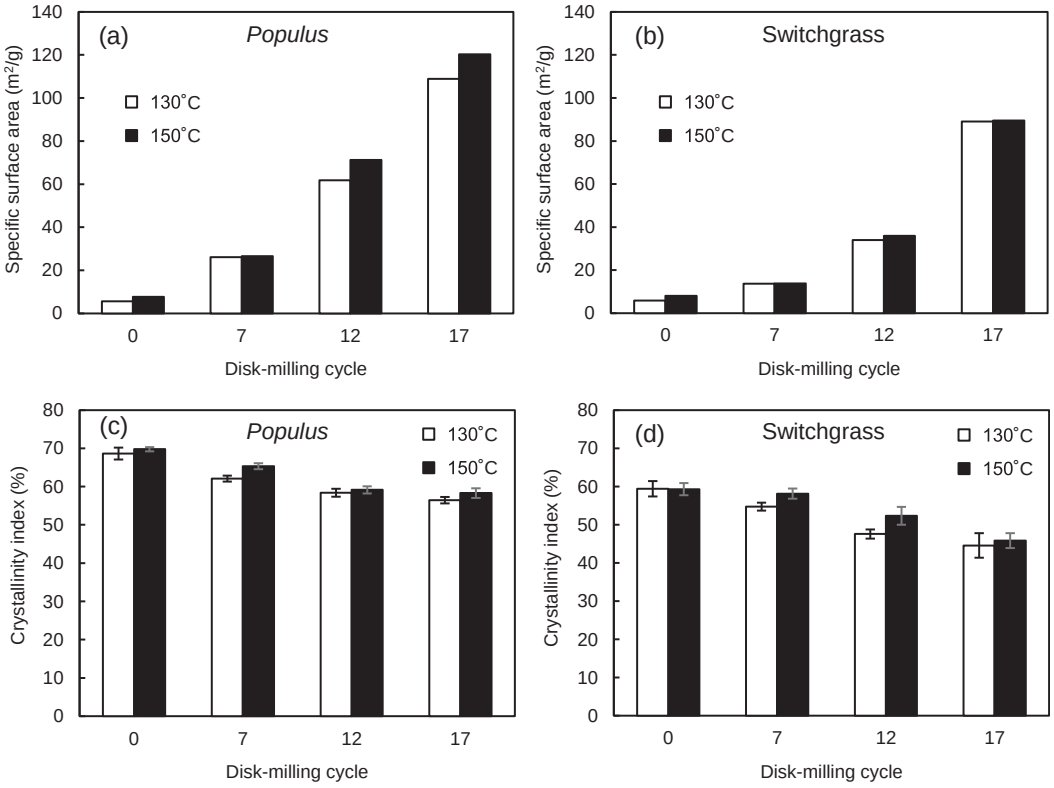

Fig. 4. (a, b) Specific surface area (SSA) and (c, d) crystallinity index (CrI) of (a, c) *Populus* and (b, d) switchgrass treated by steam treatment (ST) and wet disk milling (WDM). White boxes represent samples steam-treated at 130 °C, and black boxes represent samples steam-treated at 150 °C.

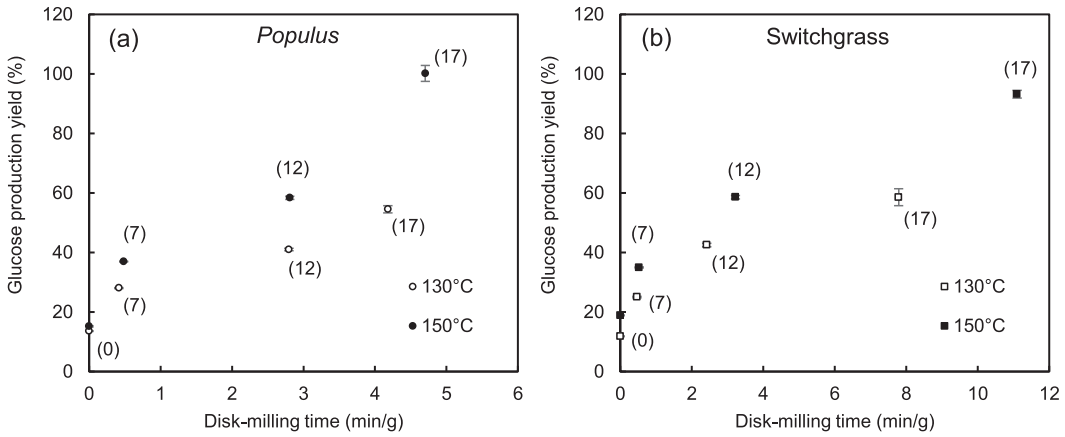

Fig. 5. Effect of disk-milling time on glucose production yield from (a) *Populus* and (b) switchgrass. White and black markers indicate steam treatment temperatures of 130 and 150 °C, respectively. The number in parentheses indicates the disk-milling cycle.

increased with disk-milling time. Gradual improvements in enzymatic saccharification by WDM alone have been reported by Hideno et al. [33] and Barros et al. [40] using rice straw and sugarcane bagasse and straw, respectively. The gradual increases in glucose yields by WDM alone reached equilibrium at 80% glucose liberation. Glucose production yields greater 80% have been achieved by combining WDM combined with other pretreatments. For example, Lee et al. [22] reported that the glucose production yield of *Eucalyptus* subjected to WDM followed by HCWT at 180 °C was 101.7%; Hideno et al. [29] reported that the glucose production yield of rice straw subjected to WDM followed by HCWT at 135 °C was 94%; and Barros et al. [40] reported that the glucose production yield of sugarcane bagasse and straw subjected to WDM followed by ozonolysis were 81.1% and 92.4%, respectively. In this study, the enzymatic saccharification of both *Populus* and switchgrass also showed great improvement when samples were subjected to ST at 150 °C and 17 cycles of WDM, and the production yields of glucose from these samples reached were 100.2% and 93.8%, respectively (Fig. 6). As shown in Fig. 7, all saccharification of pretreated samples initially proceeded rapidly and achieved equilibrium after approximately 24 h. Initial saccharification rates were positively correlated with final glucose production yields. Both *Populus* and switchgrass samples treated at 150 °C had higher initial saccharification rates than those samples treated at 130 °C and had greater than 90% glucose yields after 24 h. These results suggest that mild-temperature ST at 150 °C, combined with sufficient WDM, can be used to achieve high glucose production yields without regard to plant species.

It has been reported that SSA and CrI play important roles in the enzymatic saccharification of lignocellulosic biomass, and that biomass samples with high SSA and low CrI can be hydrolyzed most efficiently [39,41]. In fact, the glucose production yields of steam-treated samples in this study increased with increasing SSA and decreasing CrI, which were associated with disk-milling time. Endo [42] suggested that WDM can increase the available surface area for enzyme adsorption without the reducing crystallinity through fibrillation and the reduction of fiber thickness. Figs. 4 and 5 indicate that the glucose yields of enzymatic saccharification were more closely correlated with SSA than with CrI. Changes in SSA and CrI with increasing disk-milling cycle further indicate that WDM improved the reactivity of enzymatic saccharification irrespective of plant species and the temperature conditions of ST.

In this study, ST was conducted under mild-temperature conditions, and the morphology, CrI, and SSA of both *Populus* and switchgrass treated by WDM was similar at each disk-milling cycle, regardless of ST temperature conditions (Figs. 3 and 4). On the other hand, sugar production yields were clearly affected by ST temperature (Figs. 5–7). Table 2 indicates that even though ST had little effect on the chemical composition of samples, the amount of sugar elution was different for samples steam-treated at 150 °C than for

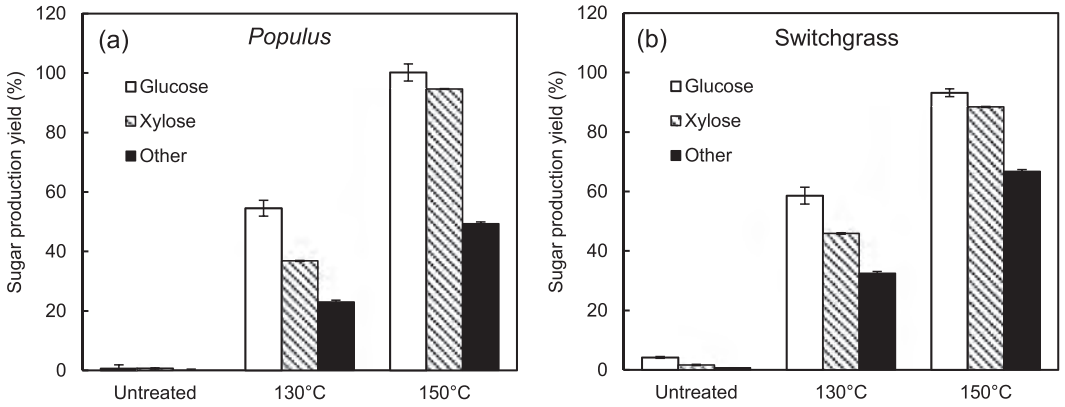

Fig. 6. Sugar production yield of 72-h enzymatic saccharification of (a) *Populus* and (b) switchgrass treated by combination of steam treatment (ST) and 17 cycles of wet disk milling (WDM). White boxes and diagonal stripes represent the production yields of glucose and xylose, respectively. Black boxes represent the production yield of mannose for *Populus* and arabinose for switchgrass samples.

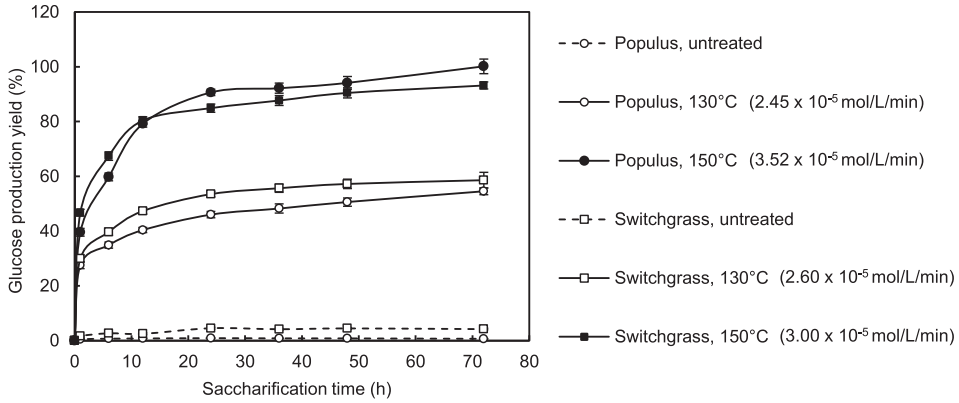

Fig. 7. Comparison of the enzymatic saccharification time profiles of *Populus* and switchgrass treated by combination of steam treatment (ST) and 17 cycles of wet disk milling (WDM). Circle and square markers indicate *Populus* and switchgrass, respectively. Dashed lines indicate untreated biomass. Solid lines with white and black markers indicate ST temperatures of 130 and 150 °C, respectively. Numeric values enclosed in parentheses next to graph legends indicate initial rates of enzymatic saccharification.

those treated at 130 °C (Table 1). More sugars were eluted from switchgrass samples steam-treated at 150 °C than from those treated at 130 °C. The data in Tables 1 and 2 indicate that the main hemicellulose structures were not greatly depolymerized by ST at 130 °C, but partial solubilization of minor portions of hemicellulose, like side chains on hemicellulose backbones, occurred. Partial removal of hemicellulose components was not observable by SEM and there was no dramatic effect on SSA and CrI, but the specific enzymatic saccharification of steam-treated samples improved significantly relative to untreated samples. The removal of sterically bulky side chains from hemicellulose is an important effect of pretreatment because the side chains may interfere with hydrolysis of hemicellulose backbone components by hemicellulases such as mannase and xylanase [43]. The removal of side chains likely promotes the digestion of hemicellulose and the subsequent degradation of cellulose. Differences in sugar yields between samples steam-treated at 130 °C and those treated at 150 °C likely result from differences in the improvement of enzymatic accessibility after ST.

Different kinds of lignocellulosic feedstocks contain different levels of three main structural components (cellulose, hemicelluloses, and lignin) and have complex differences in structure. Different in biomass compositions and overall structures require different strategies for pretreatment processing in order to effectively reduce the biomass recalcitrance of each individual feedstock. For example, based on their analyses of specific cell wall components, DeMartini et al. [44] reported that the removal of hemicellulose, especially xylan, effectively reduced switchgrass recalcitrance, whereas reducing the lignin content had the most beneficial effect in *Populus*. However, the combined pretreatment with ST and WDM used in the present study achieved glucose production yields of greater 90% in two phylogenetically distinct energy feedstocks and used environmentally friendly, mild-temperature conditions.

5. Conclusions

To evaluate the potential of mild-temperature ST and subsequent WDM as a pretreatment for lignocellulosic feedstocks, the effects of this process were investigated using the phylogenetically different energy crop feedstocks, *Populus* and switchgrass. WDM after mild-temperature ST (150 °C) facilitated the fibrillation of both *Populus* and switchgrass, increased SSA, decreased CrI, and resulted in almost complete glucan to glucose conversion. Combining ST with WDM improved the enzymatic hydrolysis of biomass independent of plant species, demonstrating that this approach has high potential as a broadly applicable pretreatment for various kinds of lignocellulosic biomass.

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