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Effect of Hot-Pressing Temperature on the Subsequent Enzymatic Saccharification and Fermentation Performance of SPORL Pretreated Forest Biomass

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Abstract Methods to increase the energy density of biofuel feedstock for shipment are important towards improving supply chain efficiency in upstream processes. Towards this end, densified pretreated lignocellulosic biomass was produced using hot-pressing. The effects of fiber hornification induced by hot-pressing on enzymatic digestibilities of lodgepole pine and poplar NE222 wood chips pretreated by sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) were examined. Pretreated wood chips were pressed at 25, 70, 90, 110, and 177 °C. The cellulose accessibilities of the pressed and unpressed substrates were evaluated using water retention value and direct cellulase adsorption measurements. Hot-pressing below 110 °C produced a degree of hornification (DH) below 0.26 and had limited effect on cellulose accessibility and enzymatic digestibility. Hot-pressing at 177 °C produced a DH of 0.86 that substantially hornified the fibers and resulted near zero saccharification. The saccharification results were consistent with cellulose accessibility data. Ethanol fermentation studies at 18 % solids suggest that a pressing below 110 °C is preferred to reduce its effect on biofuel yield.

Keywords Biomass commoditization · Supply chain logistics · Densification · Water retention value · Cellulase adsorption · Enzymatic hydrolysis

Introduction

Economical biofuel production from lignocellulosic biomass will be facilitated by developing efficient methods to integrate the supply chain from feedstock to products [1, 2]. Feedstock logistics has a significant impact on many aspects of the process because it is a primary component of the fuel or chemical costs. In particular, this stage of the supply chain can dictate the facility scale and location, as well as the technology for conversion [3, 4]. The importance of biomass logistics is enhanced by the relatively low bulk density of original biomass. Even for woody biomass, the packing density of 75–200 kg/m^3 and a moisture content of 50 % are common [3]. Transportation efficiency can be enhanced by densification and moisture reduction. One example of how these processes can be used to improve feedstock performance and logistics is with pelletizing of lignocellulosic biomass for biopower and combined heat and power (CHP) plants where pelletizing has facilitated a worldwide market of wood pellets for European power plants [5]. Recent studies [6–8] have demonstrated that this practice can also be used prior to transporting feedstock to biochemical conversion facilities without negatively impacting bioconversion efficiencies. These studies confirm a general understanding from wood fiber science that densification of untreated lignocelluloses does not influence the recalcitrance of biomass or produce fiber hornification, i.e., the irreversible collapse of fiber pores

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due to hydrogen bonding observed in chemically produced fibers upon pressing or drying [9–11].

Recently, a concept of a "preprocessing depot" or "distributed preprocessing" has been proposed as a means of enhancing the supply chain efficiency of a centralized biorefinery [2, 4, 12]. This concept can be supported by the fact that typical petroleum refineries are approximately an order of magnitude larger than the biorefineries typically envisioned. This increased facility size benefits from the economy of scale, which is limited in a biorefinery by feedstock logistics. The concept of distributed production utilizing depots has the potential to reduce costs especially if existing facilities might be used to limit capital costs. For instance, pulp mills are often discussed as the basis for forest biorefineries, but these companies do not necessarily possess the core competencies for conducting fermentation or catalytic conversion operations nor are they necessarily well versed to market to either the fuels or chemical markets. In contrast, pulp companies are well equipped and staffed to perform the critical pretreatment step in the supply chain, thereby positioning them well as a potential "preprocessing depot". To support this effort, existing corn ethanol plants are well positioned and incentivized to saccharify and ferment the pretreated biomass supplied by a preprocessing depot to produce cellulosic ethanol or biobutanol. Such a distributed preprocessing concept has potential to substantially reduce the risk of commercializing cellulosic biofuels by using existing facilities to transition into the second generation biobased fuels and chemicals.

One key step to realize this vision of decentralized production is the preparation and transport of pretreated biomass to central biorefineries. Here, the pretreated material must be dewatered and densified into unit forms that might be effectively handled, stored, and transported, all the while maintaining the form and end performance. Looking to other industries as analogs, the densified material might take the form of either pellets, briquets, or sheets. Limited studies have addressed the performance of material that has been pretreated, densified, and stored prior to enzymatic hydrolysis. One recent study suggested that pelletizing ammonia fiber expansion (AFEX)-treated corn stover at 70 °C did not substantially reduce enzymatic saccharification efficiency [13]. This result seems to contradict the current understanding of the deleterious effects of drying and pressing on subsequent enzymatic saccharification [9,10]. Pelletizing utilizes both heat and pressure to increase the energy density of the lignocellulosic biomass. Our previous study revealed that hornification reduced the performance of pretreated feedstock during enzymatic saccharification. This deterioration of performance not only depends on the exposure temperature, time, and pressure but also varies with substrate [9, 10]. Therefore, it is conceivable to achieve limited reductions in enzymatic saccharification by choosing a set of proper conditions for densifying a given feedstock. The objectives of the present study are to evaluate the potential effect of densification using hot-pressing on enzymatic saccharification of two sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) [14] pretreated wood samples. SPORL was chosen for its robust performance for forest biomass bioconversion [15, 16].

Materials and Methods

Materials

Mountain pine beetle-killed lodgepole pine trees were collected from Colorado. The trees were dead for approximately 8 years and were laid on the ground as wind fall. The logs were debarked on the site and wrapped in plastic bags and then shipped to the USDA Forest Service, Forest Products Laboratory (FPL), Madison, WI. Logs of the poplar clone, NE222, were similarly collected from Northern Wisconsin. Both types of wood biomass were chipped at FPL using a Knife chipper (Carthage (CEM) Machine Co, Carthage, New York). The wood chips were subsequently screened to remove particles larger than 38 mm and less than 6 mm. The thicknesses of the accepted chips were approximately between 1 and 5 mm. The chips were kept frozen at −16°C until use.

Commercial cellulase enzymes Cellic®CTec3 (abbreviated CTec3) were generously provided by Novozymes North America (Franklinton, NC, USA). The cellulase activity is 217 FPU/mL as calibrated [17]. Sodium acetate, acetic acid, sulfuric acid, and sodium bisulfite were used as received from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were ACS reagent grade.

SPORL Pretreatment

SPORL pretreatments of poplar NE222 (abbreviated as NE2) and beetle-killed lodgepole pine (BKLP) chips were conducted in a 390-L pilot-scale rotating wood-pulping digester. The digester was mounted on a deck approximately 3 m above ground. It was heated by a steam jacket and rotated 360° at 2 rpm during pretreatment. Wood chips (40 kg in oven dry weight) were pretreated using a dilute sulfite solution at a liquor-to-wood ratio of 3(L):1(kg). Sodium bisulfite and sulfuric acid charges in oven-dried wood were 3 % (*w/w*) and 1.1%(*w/w*) for NE2 and 8 % (*w/w*) and 2.2 % (*w/w*) for BKLP respectively. NE2 and BKLP wood chips were pretreated at 160 °C for 40 min and at 165 °C for 65 min, respectively, with a heat-up period for temperature ramping of approximately 30 and 35 min. At the end of each pretreatment, the pretreated materials were blown to a tank and cooled. Noncondensibles such as SO_2 were wet-scrubbed by a sodium hydroxide spray. Both the wet solids and pretreatment spent liquor were collected and weighted for mass balance analysis.

Hot-Pressing and Substrate Production

Following the pretreatment, the resulting wet wood chips (unwashed)were hot-pressed into a panel or sheet as an initial evaluation of the effects of heat and pressure on enzymatic hydrolysis. OD weight of 1 kg wet wood chips was formed into a mat on a perforated caul plate using a 25.4×25.4 cm forming box. The mat was then loaded into a Nordberg Manufacturing hot press (Milwaukee, WI, USA) with a PressMAN control system (Alberta Research Center, Alberta, Canada) and placed between two perforated caul plates and wire screens to allow water or steam to escape. The mats were pressed at 25 (room temperature), 70, 90, 110, or 177 °C platen temperature for 10 min at a pressure of 2.8 MPa (400 psi) based on the initial mat size. The press required approximately 30 s to close and was opened after approximately 30 min to allow any remaining steam to slowly escape when pressing at high temperatures. The final mat/board size was approximately 28 × 28× 1 cm. Three batch runs of hot-pressing were conducted under each set of conditions, and the pressed wood chips were mixed to make up enough material for the study. Table 1 listed the press conditions and the moisture contents of the biomass before and after pressing. The reported moisture contents were averages of three test samples through oven-drying at 105 ℃.

The unpressed and hot-pressed samples were reduced in size using a Waring blender (Model LB-1, Waring Products Co., Winsted, CT, USA) at solids loading of 5 % for 4 min at the high speed setting. Each resultant suspension was filtered to dewater to approximately 25 % solids and directly used as substrate for enzymatic hydrolysis and fermentation. The chemical compositions of the samples were analyzed. No noticeable differences were observed among the pressed and unpressed samples for a given wood species of NE2 or BKLP, suggesting that pressing did not affect substrate chemical composition. The averages of the pressed and unpressed samples for each wood species were reported in Table 2.

Table 1 List of press conditions and the moisture contents of the wood chips before and after press

Press T (℃)	Moisture (%)		DH after pressing
	Before pressing	After pressing	
Poplar NE2			
RoomT (≈25)	62	46	0.002
70	62	37	0.004
90	62	26	0.11
110	62	22	0.26
177	62	0.2	0.86
Lodgepole pine BKLP			
RoomT (≈25)	69	44	−0.15
90	69	26	0.26
110	69	21	0.25

WRV Measurements

The water retention value (WRV) of a substrate can be used to represent the total pore volume and, therefore, the accessibility of a lignocellulosic substrate to cellulase [9]. The WRVs of all substrates were measured following Scandinavian test method SCAN-C 62:00 [18]. Substrate solids of approximately 15 % were wrapped in a nylon mesh with 100-mm openings (Cole-Parmer, Vern on Hills, IL) and placed into a centrifuge tube with support to make space for water accumulation during centrifuging. The wrapped suspension was centrifuged at 2,000g for 15 min in a laboratory centrifuge (Thermo Fisher Scientific, Sorvall Legend 40/40R, Waltham, MA). WRV of the substrate is simply the amount of water retained after centrifuging as a percentage of the substrate dry weight. The mean of duplicate measurements was reported. The standard deviations were used as error bars in plots.

Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted at 2 % solids (*w/v*) in 50 mmol/L acetate buffer of pH 5.5 with 50 ppm tetracycline as antibiotic. An elevated pH of 5.5 can reduce non-productive cellulase binding to lignin to enhance saccharification [19, 20]. CTec3 loading was 8.0 FPU (0.037 mL) and 6.9 FPU (0.032 mL)/g glucan for NE2 and BKLP, respectively. The substrate suspension was incubated on a shaker (Thermo Fisher Scientific, Model 4450) at 50 ℃ and 200 rpm. Duplicate hydrolysis runs were conducted. The averages were reported. The standard deviations were used as error bars in plot. Glucose in the hydrolysate was determined using a commercial biochemistry analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA).

Cellulase Binding

Substrate accessibility to cellulase was also evaluated by measuring the amount of CTec3 binding to the substrate. Substrate suspension of 50 mL at 2 % (*w/v*) solids with acetate buffer of 50 mmol/L and pH 5.5 were mixed in a flask on a

Table 2 Chemical compositions of the washed pretreated NE2 and BKLP

Sample	NE2	BKLP
KLignin	24.8±0.5	33.6±0.9
Glucan	62.5±2.2	58.2±2.4
Arabinan	ND	ND
Galactan	ND	0.3±0.0
Xylan	4.4±0.5	2.8±0.3
Mannan	1.9±0.1	2.1±0.4

shaker (Thermo Fisher Scientific, Model 4450) at 200 rpm and 25 °C. CTec3 loadings were 1.73 mg protein (0.018 mL) and 2.11 mg protein (0.023 mL)/g solids substrate for NE2 and BKLP, respectively. These loadings were the same as those used for enzymatic hydrolysis study. After approximately 0.5 h, an aliquot of sample was taken and analyzed for protein using the Bradford method. Again, the mean of duplicate measurements was reported. The standard deviations were used as error bars in plots.

Fermentation

The unpressed and pressed at 110 °C pretreated NE2 and BKLP substrates were used for enzymatic saccharification and fermentation at solid content 18 %. Hydrolysis was conducted in a flask on a shaker at pH 5.5, 50 °C, and 200 rpm in 50 mmol/L acetate buffer using CTec3 at 10 FPU (0.046 mL) and 15 FPU (0.069 mL)/g glucan for NE2 and BKLP, respectively. When the solids were liquefied, the biomass slurries were cooled down to 35 °C for fermentation using *Saccharomyces cerevisiae* YRH400.

As described previously [21], the yeast YRH400 was first grown on yeast extract peptone dextrose (YPD) agar plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar at 30 °C for 2 days. A colony from the plate was transferred by loop to liquid YPD medium in a flask and cultured at 30 °C with agitation at 90 rpm on a shaking bed incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) until optical density at 600 nm reached a constant value. The yeast biomass was concentrated by centrifuge at 3,000g for 5 min and then removing the supernatant. The concentrated yeast was applied for fermentation with an initial optical density (600 nm) of 3. Duplicate fermentation runs were conducted for each substrate. Each fermentation was carried out at 90 rpm for 96 h. Samples were taken periodically for glucose and ethanol analysis in duplicate. The averages of duplicate fermentation runs were reported. The standard deviations were used as error bars in plots.

Results and Discussion

Effect of Pressing Temperature on Substrate Accessibility to Cellulase

In pelletizing woody biomass, the die temperature often varied in a range of 35–110 °C [22]. We evaluated a pressing temperature range of 25–110 °C along with an extreme temperature of 177 °C. Different pressing temperatures produced different degrees of fiber hornification, which resulted in varied substrate water swelling abilities and accessibilities to cellulase. Hot-pressing reduced WRV for both NE2 and BKLP (Fig. 1a) in agreement with our previous wet-pressing study [10]. The reduction in WRV was not substantial for substrates pressed at temperatures below 90 °C (the data point for BKLP pressed at 25 °C may be an outlier, repeated measurements showed similar results). However, the reduction was substantial at high temperatures, e.g., WRV was reduced from approximately 150 % for the unpressed substrate to 20 % when pressed at T=177 °C for NE2. The substrate was substantially hornified at this temperature. We can define the degree of hornification (DH) as the reduction in WRV as percentage of the WRV reduction for the completely hornified substrate [23]. Therefore, DH= 1 for the completely hornified substrate and DH = 0 for the never pressed substrate. As listed in Table 1, the DH of the NE2 pressed at 177 °C was 0.86. DH was only 0.11 and 0.26 for the NE2 and BKLP substrates pressed at 90 °C, respectively. The negative DH for BKLP pressed at 25 °C is an outlier, as discussed above.

WRV is a measure of the total substrate pore volume or surface. We quantified substrate accessibility by directly

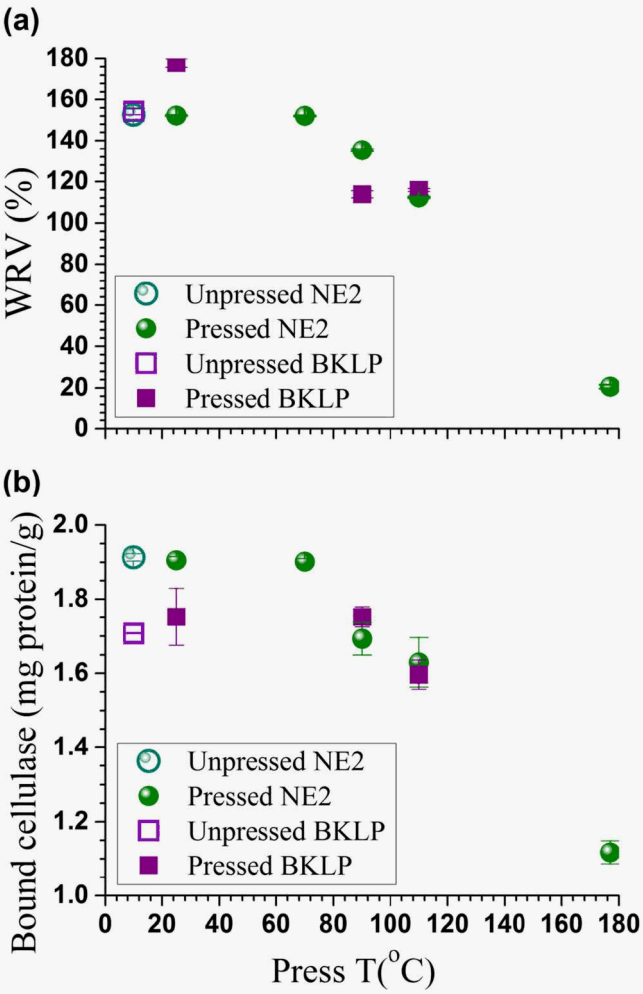

Fig. 1 Effects of hot-pressing temperature on substrate accessibility measured by **a** water retention value (WRV) and **b** cellulase adsorption (binding)

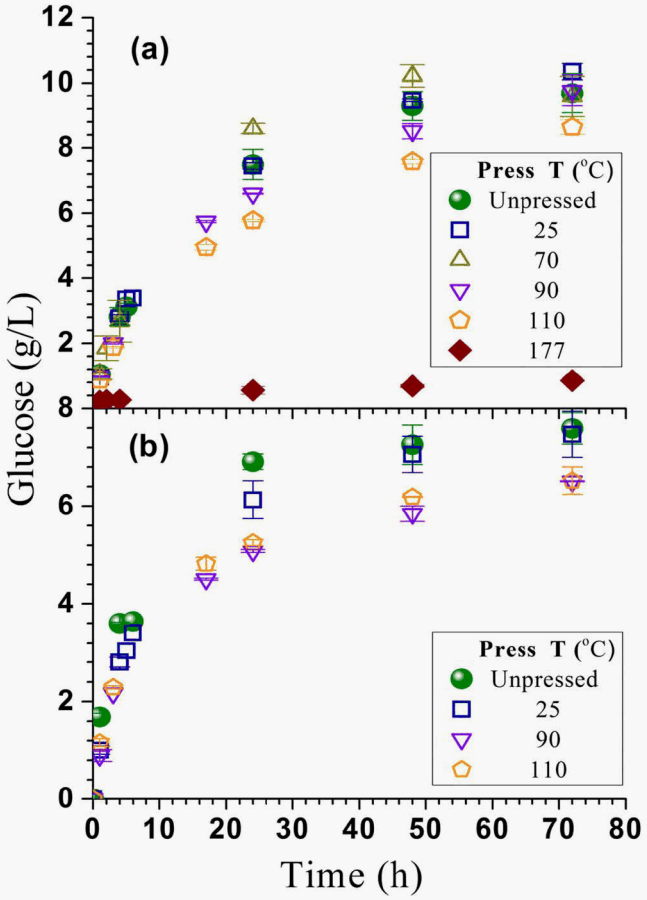

Fig. 2 Effects of hot-pressing temperature on enzymatic saccharification at 2 % solids measured by glucose concentration. **a** Poplar NE2, glucan content 62.5 %; **b** beetle-killed lodgepole pine BKLP, glucan content 58.2 %

measuring the amount of cellulase binding. Interpretation of the results indicates that the amounts of CTec3 bound to either NE2 or BKLP decreased with the increase in pressing temperature (Fig. 1b). The reduction in cellulase binding was low at low temperatures (<90 °C), but increased linearly with temperature at high temperatures. The amount of cellulase binding for NE2 pressed at 177 °C was reduced from 1.9 to 1.1 mg protein/g or a reduction of approximately 40 %. The reduction was only 11 % when NE2 was pressed at 90 °C. This behavior was similar to what was observed from WRV measurements, suggesting that substrate hornification by hot-pressing reduced substrate accessibility to cellulase especially at high temperatures.

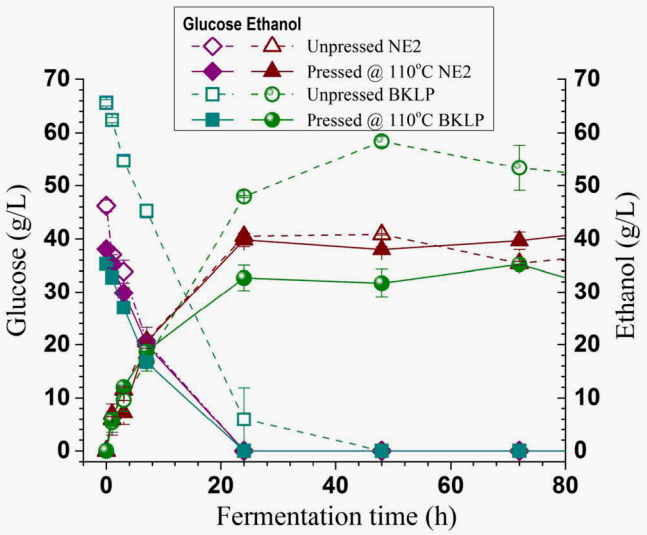

Fig. 3 Comparisons of ethanol concentration in fermentation broth between runs using unpressed and pressed at 110 °C substrates

Effect of Pressing Temperature on Enzymatic Saccharification

The time-dependent glucose concentrations in the enzymatic hydrolysates of the pressed substrates under different temperatures were compared with the hydrolysate of the unpressed substrate for both NE2 and BKLP respectively. When NE2 was pressed at 177 °C, the glucose concentration was less than 1 g/L compared with approximately 10 g/L for the unpressed substrate (Fig. 2a), a reduction by approximately an order of magnitude. When pressing temperature was reduced to 110 °C or lower, the effect of pressing on glucose concentration was not differentiable before 10 h of hydrolysis. Increasing pressing temperature to 90 or 110 °C reduced glucose concentration, as can be seen from the data taken at hydrolysis time 18–48 h (Fig. 2a). However, the reductions in glucose concentration became less obvious at 72 h. As a matter of fact, the glucose concentration of the hydrolysate derived from the substrate pressed at 90 °C was the same as that of the unpressed substrate. It is possible that longer hydrolysis time can reduce the effect of substrate accessibility. Similar results were also obtained for the BKLP sample, i.e., the difference in glucose concentration in the enzymatic hydrolysates between the substrate pressed at 90 or 110 °C, and the unpressed substrate is most obvious at hydrolysis time 24–48 h (Fig. 2b). The difference became smaller at 72 h. Overall, the enzymatic hydrolysis results are consistent with the WRV

Table 3 Comparisons of the performance of SSF using unpressed and pressed at 110 °C SPORL pretreated BKLP

Sample	Unpressed	Pressed at 110 °C
Average fermentation performance measure in the first 7 h		
Ethanol productivity	2.62±0.50	1.90±0.06
Glucose consumption	−3.07±0.27	−2.67±0.05
Terminal maximal ethanol production at 72 h		
Ethanol concentration (g/L)	50.6±5.4	27.8±1.9
Ethanol yield (g/g sugar)[a]	0.419±0.044	0.230±0.025

[a] Based on the glucan and mannan content in the washed pretreated solids used in fermentation

and cellulase-binding data, i.e., pressing wood chips at temperatures below 90 °C or even 110 °C only caused small reductions in substrate accessibility and enzymatic saccharification efficiency.

Comparisons of Ethanol Production from the Unpressed and Pressed (110 °C) Substrates

Ethanol concentrations in the fermentation broths suggest that hot-pressing at 110 °C did not reduce ethanol yield for NE2, but substantially reduced ethanol yield for BKLP (Fig. 3). The fact that a slightly lower initial glucose concentration in fermenting the pressed NE2 did not reduce ethanol yield suggests continued saccharification during fermentation was able to make up the difference. In other words, a small reduction in substrate accessibility may not affect final biofuel yield through fermentation. The initial glucose concentration at the end of liquefaction period (t=0, Fig. 3) of the pressed (at 110 °C) BKLP was substantially lower (by 50 %) than that of the unpressed BKLP, which is different from that observed at low solids saccharification study (Fig. 2b). This indicates that saccharifying at low solids did not provide the complete picture of the effect of hot-pressing on biomass bioconversion. The substantially low glucose concentration may have affected both glucose consumption and ethanol productivity in early stage of fermentation (Table 3). The reduction in ethanol productivity (27 %) is much greater than the reduction of glucose consumption (13 %). However, the lack of continued robust saccharification of the pressed (at 110 °C) BKLP during fermentation due to hot-pressing induced fiber hornification also contribute to the substantially lower (by 45 %) terminal (72 h) ethanol concentration and yield than those of the unpressed BKLP (Table 3).

Conclusions

Densification of pretreated lignocelluloses to facilitate biomass logistics can produce fiber hornification to reduce substrate accessibility to cellulases and, therefore, enzymatic saccharification yield. Under the mild pressing temperature, e.g., below 70 °C, the reduction in cellulose accessibility is negligible. Therefore, this effect of densification can be managed by using a relatively low temperature. The densification effect also depends on the enzymatic digestibility of the initial pretreated feedstock in addition to pressed temperature, pressure, and time. Fermentation results indicate that for the SPORL pretreated lodgepole pine, a temperature lower than 110 °C may be preferred to reduce the effect of densification to maximize biofuel yield. However, pressing at 110 °C of the SPORL pretreated poplar NE222 had no effect on terminal ethanol yield. Future study using real pellets is needed for process design and economic analysis.

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