Using a combined hydrolysis factor to optimize high titer ethanol production from sulfite-pretreated poplar without detoxification

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HIGHLIGHTS

- Combined hydrolysis factor for optimizing sulfite pretreatment.
- High ethanol titer at 41 g/L from poplar by sulfite without detoxification.
- Low cellulase loading of 15 FPU/g glucan (27 mL/kg wood).

GRAPHICAL ABSTRACT

ABSTRACT

Sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) was applied to poplar NE222 chips in a range of chemical loadings, temperatures, and times. The combined hydrolysis factor (CHF) as a pretreatment severity accurately predicted xylan dissolution by SPORL. Good correlations between CHF and pretreated solids enzymatic digestibility, sugar yield, and the formations of furfural and acetic acid were obtained. Therefore, CHF was used to balance sugar yield with the formation of fermentation inhibitors for high titer ethanol production without detoxification. The results indicated that optimal sugar yield can be achieved at $\text{CHF} = 3.1$, however, fermentation using un-detoxified whole slurries of NE222 pretreated at different severities by SPORL indicated CHF = 2 produced best results. An ethanol titer of 41 g/L was achieved at total solids of approximately 20 wt% without detoxification with a low cellulase loading of 15 FPU/g glucan (27 mL/kg untreated wood).

1. Introduction

Short rotation woody crops such as *Populus* spp. and their hybrids (i.e., hybrid poplars) are a significant component of the total biofuels and bioenergy feedstock resource in the USA and are, therefore, vital for growing a bioeconomy. An attractive aspect of growing hybrid poplars is their ability to grow on marginal lands to conserve water, recycle nutrients, and sequester carbon (Vance et al., 2010). However, production of these dedicated energy crops on such marginal and liability lands may lead to questions about their economic, logistic, and ecologic feasibility. In this context, conversion efficiencies at the back end of the energy supply chain are of utmost importance. Despite many research efforts have been made in bioconversion of hybrid poplars (Acker et al., 2014; Gupta

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et al., 2014; Kim et al., 2013; Kundu et al., 2014; Wang et al., 2012; Wyman et al., 2009), achieving high titer and yield biofuel production through fermentation from poplars without detoxification remains a challenge.

As a woody biomass, poplars can be highly recalcitrant to enzymatic saccharification depending on its lignin content and structure (Studer et al., 2011; Wang et al., 2012). Severe pretreatments to remove this recalcitrance often result in substantial amount of sugar (especially xylose) degradation to furans (furfural) that can inhibit fermentation at high solids loadings for high titer biofuel production. Furthermore, unlike softwood species, poplar woods also contain a large amount of acetyl groups that can be easily converted into acetic acid by acidic pretreatments (Tian et al., 2011; Tunc and Van Heiningen, 2008) to inhibit fermentation (Casey et al., 2010; Helle et al., 2003). Acetic acid cannot be metabolized by yeasts (Wei et al., 2013). Furthermore, in-process reduction of acetic acid formation is difficult unless alkaline pretreatments were used. De-acytlylation using hydroxide can reduce acetic acid formation (Chen et al., 2012; Kundu et al., 2014) but at the expense of additional processing. Post-pretreatment detoxification of acetic acid is also difficult as acetic acid cannot be easily neutralized or distillated (Xavier et al., 2010). The compounding effects of fermentation inhibition by furans and acetic acid along with aromatics (Palmqvist et al., 1999; Pampulha and Loureiro-Dias, 1989) make high titer biofuel production from poplar woods without detoxification difficult. (Casey et al., 2010; Helle et al., 2003). Acetic acid cannot be metabolized by yeasts (Wei et al., 2013). Furthermore, in-process reduction of acetic acid formation is difficult unless alkaline pretreatments were used. De-acytlylation using hydroxide can reduce acetic acid formation (Chen et al., 2012; Kundu et al., 2014) but at the expense of additional processing. Post-pretreatment detoxification of acetic acid is also difficult as acetic acid cannot be easily neutralized or distillated (Xavier et al., 2010). The compounding effects of fermentation inhibition by furans and acetic acid along with aromatics (Palmqvist et al., 1999; Pampulha and Loureiro-Dias, 1989) make high titer biofuel production from poplar woods without detoxification difficult.

Here we demonstrate high titer ethanol production from an undetoxified whole slurry of poplar wood pretreated by SPORL (sulfite pretreatment to overcome the recalcitrance of lignocellulosics). SPORL has demonstrated robust performance in pretreating softwoods for high titer and high yield bioethanol production without detoxification (Zhou et al., 2013; Zhu et al., 2015). The relatively lower furan formation by SPORL compared with dilute acid pretreatment (Shuai et al., 2010; Wang et al., 2009) can alleviate the problem of the compounding effect of fermentation inhibition. Therefore, optimization of SPORL with the aim to reduce this compounding effect on cell growth can be effective. Reducing pretreatment severity such as using a low pretreatment temperature or a low acid concentration can reduce xylen dissolution, acetic acid formation, and sugar degradation to furans. To address the deficiency in dissolving hemicelluloses at low temperatures or low acid conditions for efficient enzymatic saccharification of cellulose, we extended the pretreatment duration to maintain a sufficient pretreatment severity measured by a combined hydrolysis factor (CHF) that can accurately predict dissolution of hemicelluloses (Zhou et al., 2013; Zhu et al., 2012). This approach of pretreatment optimization differs from traditional statistical experimental designs. It also differs from other low temperature pretreatment studies that arbitrarily selected pretreatment duration (Chen et al., 2012). The objective of this study was to demonstrate pretreatment optimization using CHF to achieve in-process inhibitor reduction for high titer ethanol production from a poplar wood without post-pretreatment detoxification. The results can be used to enhance the sustainability of these conversion systems, especially with respect to the integration of optimization at the front end (i.e., during pretreatment) and the elimination of unnecessary steps thereafter (i.e., not needing post-pretreatment detoxification). Therefore this study is important for researchers and industrial representatives seeking to increase efficiencies in ethanol production from wood.

2. Methods

2.1. Materials

Wood logs of poplar NE222 (Populus deltoides Bartr. ex Marsh × Populus nigra L.) were harvested from Hugo Sauer Nursery in Rhinelander, WI, USA, and provided by the Institute for Applied Ecosystem Studies of the USDA Forest Service Northern Research Station. The logs were transported to the USDA Forest Products Lab, Madison, WI and chipped using a knife chipper (Carthage (CEM) Machine Co, Carthage, New York). The wood chips were screened to remove particles larger than 38 mm and less than 6 mm. The thicknesses of the accepted chips ranged from 1 to 5 mm. The moisture content of the accepted wood chips was 51.6%. The chips were kept frozen at −16 °C until use.

A commercial cellulase enzyme Cellic®Ctec3 (abbreviated Ctec3) was complimentarily provided by Novozymes North America (Franklin, NC, USA). The cellulase activity was 217 FPU/ml calibrated using a literature method (Wood and Bhat, 1988). Sodium acetate, acetic acid, sulfuric acid, and sodium bisulfite were ACS reagent grade and were acquired from Sigma-Aldrich (St. Louis, MO, USA).

Saccharomyces cerevisiae YRH400, an engineered fungal strain (Hector et al., 2011), was provided by USDA Agriculture Research Service for fermentation of xylose and hexoses. The strain was grown at 30 °C for 2 days on YPD agar plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar. A colony from the plate was transferred by loop to a liquid YPD medium and cultured in a flask overnight at 30 °C on a shaking bed incubator at 90 rpm (Thermo Fisher Scientific, Model 4450, Waltham, MA). The cultured medium was used as inoculant for fermentation.

2.2. Pretreatment

Poplar NE222 wood chips of 150 g in oven dried (OD) weight were placed in a 1-L reactor with a dilute sulfite solution at wood (OD) to liquor ratio W:L = 1:3 (kg/L) or total solids loading of 25 wt%. Three 1-L reactors were placed into a 23-L rotating wood chipper (Carthage (CEM) Machine Co, Carthage, New York). The wood was kept at 4 °C until...
Table 1

List of pretreatment conditions conducted at liquor to wood ratio L/W = 3:1 (L/kg), along with the calculated combined hydrolysis factors (CHF).

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<tr>
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<th>Sulfuric acid in liquor (mL/L)</th>
<th>Sodium bisulfite on wood (%)</th>
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<th>Ramping time (min)</th>
<th>Time (min)</th>
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a Tx stands for pretreatment temperature: 175, 160, 150, 135 °C; Ax stands for sulfuric acid concentration in initial liquor in mL/L; txxx stands for pretreatment duration at T in min; Rx is pretreatment replicate number.
b Combined hydrolysis factor is defined according to Eq. (2).

Fig. 1. A schematic experimental flow diagram for producing high titer ethanol from poplar wood in this study.
Monosaccharides, furfural, and 5-hydroxymethylfurfural (HMF), acetic acid, and ethanol in pretreatment spent liquor and fermentation broth samples were determined using a Dionex HPLC system (Ultimate 3000) equipped with an RI (RI-101) detector and a UV detector (VWD-3400RS), as described previously.

using a Büchner funnel with a nylon filter. The washed solids yield was determined.

2.3. Analytical methods

Small samples of untreated and pretreated poplar NE222 solids were oven dried at 105 °C overnight, then cooled down and Wiley milled to 20 mesh (model No. 2, Arthur Thomas Co, Philadelphia, PA, USA). The milled samples were hydrolyzed using sulfuric acid in two steps for carbohydrates and Klassen lignin analyses by the Analytical Chemistry and Microscopy Lab (ACML) at the Forest Products Lab as described previously. Monosaccharides, furfural, and 5-hydroxymethylfurfural (HMF), acetic acid, and ethanol in pretreatment spent liquor and fermentation broth samples were determined using a Dionex HPLC system (Ultimate 3000) equipped with an RI (RI-101) detector and a UV detector (VWD-3400RS), as described previously.

2.4. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were carried out in a 500-ml flask at solid (DM) loading of 20 g/L in a sodium acetate buffer solution of 50 mM at pH 5.5. The elevated pH of 5.5 compared to the conventional range of 4.8–5.0 was found to effectively reduce unproductive cellulase binding to lignin and, therefore, substantially enhance enzymatic saccharification (Luo et al., 2013; Lou et al., 2013; Wang et al., 2013). The flask was placed on a shaking bed (Model 4450, Thermo Fisher Scientific, Waltham, MA) at 200 rpm and temperature controlled at 50 °C. The Ctec3 loading was 10 FPU/g glucan. Tetracycline was applied (40 ppm) into each run to inhibit bacterial growth and reduce unproductive cellulase binding to lignin and therefore substantially enhance enzymatic saccharification (Luo et al., 2013; Lou et al., 2013; Wang et al., 2013). The flask was placed on a shaking bed (Model 4450, Thermo Fisher Scientific, Waltham, MA) at 200 rpm and temperature controlled at 50 °C. The CTec3 loading was 10 FPU/g glucan. Tetracycline was applied (40 ppm) into each run to inhibit bacterial growth and reduce unproductive cellulase binding to lignin and therefore substantially enhance enzymatic saccharification (Luo et al., 2013; Lou et al., 2013; Wang et al., 2013).

2.5. Quasi-simultaneous enzymatic saccharification and combined fermentation (Q-SSCombF)

Quasi-simultaneous enzymatic saccharification and combined fermentation (Q-SSCombF) of the reconstituted pretreated whole...
slurry using the pretreated solids and spent liquor were conducted in 125-mL Erlenmeyer flasks at 12.8 wt% washed DM loading (equivalent to approximately 20 wt% total DM loading). The collected spent liquor was first neutralized to pH 6.0 using lime and then added into the washed pretreated solids in proportion based on the theoretical spent liquor volume (3 L/kg OD untreated NE222) and the total yield of the washed solids to re-constitute the SPORI pretreated whole slurry of NE222 (Fig. 1). The CTeC3 loading was 15 FPU/g glucan in washed solids (equivalent to 27 mL CTeC3/kg untreated wood). Along with the application of acetic sodium acetate buffer, CTeC3 solution, the total mass in each Q-SSCombF run was 50 g (approximately 50 mL). Enzymatic liquefaction was carried out at 50 °C, pH = 5.5 to enhance saccharification (Lan et al., 2013; Lou et al., 2013; Wang et al., 2013), and 200 rpm on a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltman, MA) for 20–48 h. The liquefied sample was cooled down to 35 °C and inoculated with the YRH 400 yeast seeds at loading of 0.6 dry cell/g substrate. The shaker speed was reduced to 90 rpm. Fermentation was conducted at 35 °C. No nutrients were supplemented. Samples were taken periodically for analysis of monosaccharides, inhibitors and ethanol. Replicate fermentation runs were conducted to ensure experimental repeatability. The standard deviations were used as error bars in plotting.

3. Results and discussion

3.1. Predicting xylan dissolution using the combined hydrolysis factor (CHF)

The cell wall compositions of the pretreated NE222 solids under different pretreatment conditions were analyzed (Table 2) to calculate component yields. Xylan yields as percent of original xylan in wood (determined from xylan content and yield of washed solids), XE, from all 36 pretreatments were fitted to Eq. (1) as a function of the combined hydrolysis factor (CHF) (Zhu et al., 2012).

\[
X_R = (1 - \theta)e^{-\text{CHF}} + \theta e^{e^{-\text{CHF}}}
\]

(1)

\[
\text{CHF} = e^{(chf + k_{PA} + k_{PB})/c_{CHF}} (C_A + C_B)t
\]

(2)

Eq. (1) was derived from first order reaction kinetics when hemicelluloses (xylan in the present study) were modeled by a slow and a fast (hydrolysis) reaction fraction, i.e., \( \theta \) and \((1 - \theta)\), respectively. \( f \) is the ratio of the rate constant between slow and fast xylan; \( C_A \) and \( C_B \) are the concentrations of chemical A (H2SO4) and chemical B (NaHSO3) used in pretreatments, respectively; \( \alpha, \beta \) (L/mole), and \( \gamma \) (L/mole) are adjustable parameters, \( E \) (J/mole) is apparent activation energy, \( T \) is temperature in degree Kelvin, and \( R = 8.314 \) (J/mole/K) is the universal gas constant. The fitting of the xylan yield data to Eq. (1) as shown in Fig. 2 produced the adjusted parameters, \( \alpha, \beta, \) and \( \gamma \), along with \( E, \theta \), and \( f \) as listed in Table 3. As an exponential function, xylan dissolution was initially rapid and followed a trend of increasing pretreatment severity. Approximately 70% of the xylan was dissolved at \( \text{CHF} = 2 \) (Fig. 2). The dissolution of xylan slowed substantially at \( \text{CHF} = 4 \) with \( X_E \approx 0.15 \), suggesting the slow hydrolysis reaction of slow xylan fraction \((\theta = 0.178 \text{ from fitting, see Table 3})\). The importance of this is that the washed solid substrate enzymatic digestibility (SED), defined as the percentage of substrate glucon enzymatically saccharified into glucose, can be correlated to xylan removal or yield \( X_E \) within ±5% variability. Xylan yield \( X_E \) can be predicted well by pretreatment severity \( \text{CHF} \) (Fig. 2), therefore \( \text{CHF} \) may be used for pretreatment optimization as will be discussed in the following sections. The results presented in Fig. 2 are in agreement with a previous study using aspen (Zhu et al., 2012) though the range of \( \text{CHF} \) and corresponding xylan yield differ.

### 3.2. Optimizing sugar yield using the combined hydrolysis factor (CHF)

Enzymatic hydrolysis glucose yields (EHGY), SEDs, as well xylose concentrations in the spent liquors were plotted against \( \text{CHF} \) for process optimization. In general these sugar yield measures were correlated with \( \text{CHF} \) well even though pretreatments were conducted at different chemical loadings. As pretreatment severity increased, more xylan was removed and the cell wall became more porous and therefore more accessible by cellulase, which resulted in an improved enzymatic digestibility (Fig. 3a). When \( \text{CHF} \) reached approximately 6, xylan dissolution achieved 90% (Fig. 2). Further increasing severity resulted in minimal increment in xylan dissolution. As a result, SED improvement was minimal (Fig. 3a). Similar results were observed for EHGY (Fig. 3b). However, EHGY plateaued at a smaller \( \text{CHF} \approx 4 \) because EHGY is also dependent on glucon yield from pretreatment in addition to SED. Increasing pretreatment severity (CHF) always resulted in decreased glucon yield.

Similarly, xylose concentration in the spent liquor increased initially with greater \( \text{CHF} \) due to accelerated dissolution of xylan. However, xylose degradation becomes important as \( \text{CHF} \) was increased beyond 4 as the fast xylan fraction was completely removed \((X_E \approx 0.15 \rightarrow 0.178)\) to result in reduced xylose concentrations (Fig. 3c). A higher pretreatment temperature resulted in a lower maximal xylose concentration (Fig. 3c), suggesting xylose degradation reactions have higher activation energy than that for xylan dissolution reactions.

The data in Fig. 3a–c indicated that optimizing sugar yield using \( \text{CHF} \) is possible because of the small deviations in sugar yields in the correlation with \( \text{CHF} \). These deviations were caused by the variations in individual pretreatment conditions. For example, it

### Table 3

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<th>Parameters</th>
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<th>Unit</th>
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<tr>
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<tr>
<td>( \beta )</td>
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</tr>
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<td>( E )</td>
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</tr>
<tr>
<td>( \theta )</td>
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</tr>
<tr>
<td>( f )</td>
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</tr>
</tbody>
</table>
is known that sulfite loading can affect delignification (Zhang et al., 2014) and consequently SED. Acid loading affected pH and therefore sugar degradation. The results also indicated the robust performance of SPORL for NE222. The maximal sugar yield for both glucose and xylose can be achieved when the fast xylan was completely removed, i.e., \( X_R = 0 \). We can use this optimization criteria to determine the required pretreatment severity \( \text{CHF} = 3.1 \) from Eq. (1). At this \( \text{CHF} \), EHGY was nearly constant while SED and EHGY reached approximately 80% and 75% theoretical with a moderate CTeC3 loading of 10 FPU/g glucan (Fig. 3a and b). Xylose yield was also nearly maximized at approximately 65% theoretical based on the measured xylose concentration around 30 g/L (Fig. 3c and Table 2).

3.3. Balancing fermentation inhibitor formation and sugar yield in pretreatments

Increasing pretreatment severity not only resulted in sugar degradation to furans but also increased the formation of acetic acid as a result of improved xylan dissolution because acetyl groups are mainly from acetylated hemicelluloses, xylan for hardwoods (Gille and Pauly, 2012). The acetic acid and furfural concentrations in the spent liquor were found to increase with \( \text{CHF} \) (Fig. 4a and b). Acetic acid concentration plateaued at \( \text{CHF} = 8 \) as a result of near complete conversion of acetyl groups to acetic acid. Although using a \( \text{CHF} = 3.1 \) produced an optimal sugar yield, sugar yield should balance with inhibitor formation to maximize ethanol production without detoxification. \( S. \text{cerevisiae} \) can grow at acetic acid concentration of approximately 15 g/L or furfural concentration of approximately 3 g/L (Keating et al., 2006). As shown in Fig. 4a and b, a pretreatment with \( \text{CHF} = 2 \) produced approximately 15 g/L acetic acid and 1 g/L furfural in the spent liquor. The concentrations of these two inhibitors in the fermentation broth were lower because washed solids loading of 12.8 wt% is equivalent to approximately 20 wt% total solid DM loading that is below the 25 wt% total solid DM loading in pretreatments. Under the presence of multiple inhibitors, yeast tolerance to individual inhibitor is often lowered due to the compounding effects. Therefore, we suggest a \( \text{CHF} \) of approximately 2 as the

Fig. 3. Effect of pretreatment severity, \( \text{CHF} \), on (a) washed substrate enzymatic digestibility (SED), (b) enzymatic hydrolysis glucose yield (EHGY), and (c) xylose concentration in the pretreatment spent liquor.

Fig. 4. Effect of pretreatment severity, \( \text{CHF} \), on the formation of (a) acetic acid and (b) furfural, measured as concentrations in spent liquors.
optimal pretreatment severity to facilitate fermentation of the reconstituted whole slurry without detoxification. While $CHF \approx 2$ was not optimal for sugar production (Fig. 3a–c), approximately 70% of the xylan was removed (Fig. 3) to result in a reasonable sugar yield at certain pretreatment conditions. For example, EHGY was over 320 g/kg NE222 wood and SED reached 70% for run T3A2B4t290 (Fig. 2b and Table 2) with $CHF = 1.87$.

3.4. Ethanol production from pretreated poplar at high solids without detoxification

Yeast fermentation using the reconstituted whole slurries of the pretreated NE222 under 4 pretreatment temperatures with varied pretreatment time and therefore CHF but under the same chemical loadings were conducted. The pretreatment severity CHF varied from 1.87 to 5.31. The same chemical loadings were used primarily to eliminate the effects of chemical loadings on sugar yield as discussed in Section 3.2 (Fig. 3a–c). In other words, we attempted to balance sugar yield and inhibitor formation through adjusting pretreatment temperature and time. The sulfuric acid and sodium bisulfite loadings of 2 mL/L and 4 wt%, i.e., runs with A2B4, were chosen based on previous laboratory optimization for poplar wood (Wang et al., 2012). The results indicated that ethanol yield and productivity decreased with CHF (Fig. 5) though EHGY was increased (Table 2). The pretreatment (T3A2B4t290) at the lowest temperature 135 °C with $CHF = 1.87$ produced the highest ethanol yield of 0.33 g/g polymer sugar in the whole slurry (Table 4) that was not excellent but good considering the yield loss in enzymatic saccharification and low xylose fermentation yield as discussed below.

When examining the time-dependent fermentation results (Fig. 6a–f), it was apparent that the differences in ethanol yield among the 4 different samples were due to fermentation inhibition. Sample T3A2B4t290 with the lowest pretreatment severity $CHF = 1.87$ had the greatest ethanol productivity and glucose consumption (Fig. 6a and b, Table 4). Glucose consumption was nearly completed in the first 48 h with a terminal ethanol concentration of 40.6 g/L comparing with over 100 h for T5A2B4t108 ($CHF = 2.68$). The spent liquor of T5A2B4t108 contained approximately 15% more acetic acid and 30% more furfural than the liquor of T3A2B4t290 (Table 2); as a result, complete furfural metabolization was achieved in 140 h compared with 48 h for T3A2B4t290 (Fig. 6d). However, the difference in overall ethanol yield between these two samples was only approximately 14% (Fig. 5 and Table 4). Moreover, xylose consumptions were approximately the same at 50% (Fig. 6c). Further increase in ethanol titer and therefore yield for T5A2B4t108 is very likely with extended fermentation time based on the ethanol concentration profile in Fig. 6a as glucose was just completed at the end of fermentation of 170 h (Fig. 6b). This suggests a $CHF = 1.87–2.68$ is amenable for high solids fermentation, with maximal acetic acid, furfural, and HMF concentrations at 11, 0.7, and 0.1 g/L, respectively, in the fermentation broth, in addition to significant amount of aromatics from dissolved lignin. This corroborated our suggested optimal $CHF \approx 2.0$.

When comparing samples T5A2B4t108 with T6A2B4t58 and T7A2B4t24, the compounding effects of furfural on fermentation performance became clear. These three samples had similar level of acetic acid of approximately 19.5 g/L in their spent liquors (Table 2) and approximately 10.5 g/L in the initial fermentation broth (Fig. 6f). Increasing furfural concentration to 1.6 g/L (sample T6A2B4t58) reduced glucose and xylose consumption (Fig. 6b and c, Table 4) and therefore ethanol productivity (Fig. 6a and Table 4). However, terminal ethanol concentration was not substantially reduced (Table 4). When furfural concentration was further increased to 2.0 g/L (sample T7A2B4t24), fermentation became inviable while substantial amounts of glucose and xylose were not consumed and terminal ethanol concentration was only 8.4 g/L compared with 36.6 g/L for T5A2B4t108 (Table 4).

The results shown in Figs. 5 and 6 can be misleading because higher CHFs were associated with pretreatments at higher temperatures. Good ethanol yield can be obtained from pretreatments at higher temperatures as long as an appropriate CHF was selected by using a shorter pretreatment time. Certainly a pretreatment time of 290 min at 135 °C is too long. When pretreatments are conducted at 150 and 160 °C, respectively, the appropriate pretreatment time can be determined to be 84 and 37 min (including half ramping time), respectively, based on optimal $CHF \approx 2$. Similar optimization can also be implemented using CHF with slightly higher values.
reduced acid loading. The limited data (Table 2) indicated that a low acid loading (1 mL/L, or A1) resulted in low acetic acid formation which may facilitate fermentation even with a slightly higher CHF. For example, sample T7A1B2t24 (CHF = 3.48) had a low acetic acid and equivalent furfural but higher SED than sample T5A2B4t108 (CHF = 2.68). Preliminary fermentation showed ethanol production of 0.31 g/g polymer sugar was higher than the trend with CHF shown in Table 4 and Fig. 5. Further study is needed in the future, especially with respect to optimization of chemical loading.

4. Conclusions

SPORL has the potential to address the difficulties in high titer ethanol production from hardwoods such as poplars due to the compounding effect of fermentation inhibition from furans and acetic acid. The combined hydrolysis factor (CHF) can be used to optimize ethanol production at high titer without detoxification by balancing sugar yield with inhibitor formation. An ethanol titer of 41 g/L was produced at total solids loading of approximately 20% without detoxification from poplar NE222 pretreated by SPORL at CHF = 1.87. It is expected that optimal ethanol production can be achieved at varied pretreatment conditions but with similar CHF.

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Fig. 6. Comparisons of time dependent fermentation performances among four reconstituted SPORL pretreated whole slurries of poplar NE222 without detoxification. (a) Ethanol production; (b) glucose consumption; (c) xylose consumption; (d) furfural metabolization; (e) HMF metabolization; (f) acetic acid concentration.
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.03.080.

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