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# Robust cellulosic ethanol production from SPORL-pretreated lodgepole pine using an adapted strain *Saccharomyces cerevisiae* without detoxification ☆

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ABSTRACT

This study reports an ethanol yield of 270 L/ton wood from lodgepole pine pretreated with sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) using an adapted strain, *Saccharomyces cerevisiae* Y5, without detoxification. The enzymatic hydrolysate produced from pretreated cellulosic solids substrate was combined with pretreatment hydrolysate before fermentation. Detoxification of the pretreatment hydrolysate using overliming or XAD-4 resin before being combined with enzymatic hydrolysate improved ethanol productivity in the first 4 h of fermentation and overall fermentation efficiency. However, detoxification did not improve final ethanol yield because of sugar losses. The Y5 strain showed excellent ethanol productivities of 2.0 and 0.8 g/L/h averaged over a period of 4 and 24 h, respectively, in the undetoxified run. The furan metabolization rates of the Y5 strain were significantly higher for the undetoxified run than those for the detoxidfied runs, suggesting it can tolerate even higher furan concentrations than those studied. Preliminary mass and energy balances were conducted. SPORL produced an excellent monomeric sugar recovery value of about 85% theoretical and a net energy output of 4.05 GJ/ton wood with an ethanol energy production efficiency of 178% before distillation.



## 1. Introduction

Efficient production of cellulosic ethanol from plant biomass remains a challenge, although much progress has been made in many areas of biomass biorefinery. Few pretreatment methods can produce high sugar recovery from plant biomass with low energy input (Zhu and Pan, 2010). This is especially true for woody biomass because of its strong physical and chemical recalcitrance. Acid-based pretreatments, such as dilute acid, acid-catalyzed steam explosion, ethanol organosolv, and sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) (Zhu et al., 2009), have been commonly applied in research for their effectiveness in removing plant biomass recalcitrance (Yang and Wyman, 2008; Zhu and Pan, 2010). Good enzymatic cellulose saccharification efficiency from most plant biomass has been achieved using these pretreatment methods under relatively harsh conditions. However, fermentation inhibitors, such as furfural, HMF, and acetic acid, are also formed, which significantly affects ethanol production from the hemicellulose sugar stream. As a result, most studies on woody biomass using these pretreatment methods reported ethanol production only from the solid substrate cellulose fractions due to the difficulties in fermentation of inhibitor-containing pretreatment hydrolysate consisting of primarily hemicellulose sugars (Munoz et al., 2007; Sassner et al., 2008; Wyman et al., 2009). Few studies reported complete mass balance and net energy output for the process technologies examined.

Fermentation of the hemicellulosic sugar stream in the pretreatment hydrolysate is required to provide mass and energy balance process data to demonstrate the viability of a pretreatment method for practical applications. Various detoxification techniques, such as overliming and ion exchange resin, have been commonly applied before fermentation of the pretreatment hydrolysate (Cardona et al., 2010; Xavier et al., 2010; Zhu et al., 2010b). Hydrolysate detoxification adds an extra process step in practice and can result in sugar losses. Furthermore, it is effective only to certain degrees and therefore should be avoided if at all possible. Recently, a few studies reported fermentation of pretreatment hydrolysate without detoxification by using very mild dilute

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acid pretreatments to significantly reduce formation of fermentation inhibitors (Huang et al., 2009; Scordia et al., 2010). However, maximizing ethanol production from the more important abundant fraction, cellulose, must also be realized to maximize ethanol yield through hydrolysis and fermentation of lignocelluloses. Although alkaline-based pretreatment methods can produce less toxic or non-toxic hydrolysates, these methods have their own problems for practical applications (Zhu and Pan, 2010), such as very low sugar yield from woody biomass using ammonia-based methods. It is of great practical importance to maximize both glucose recovery and ethanol yield from the cellulose fraction using robust pretreatment and to improve fermentation efficiency of the hemicellulose sugar streams without detoxification. This presents a significant challenge to cellulosic ethanol production, especially from woody biomass because of its strong recalcitrance that limits pretreatment options.

Woody biomass is an important feedstock for the future bio-based economy because of its availabilities in large quantities in many regions of the world and its advantages in transportation, storage, etc. (Zhu and Pan, 2010). Previously, we demonstrated the robust performance of SPORL pretreatment for producing readily digestible substrates from both softwood and hardwood with low energy input and low inhibitor generation (Wang et al., 2009; Zhu et al., 2009). We achieved an ethanol yield of 276 L/ton wood, or 72% theoretical yield, from lodgepole pine using a conventional *Saccharomyces cerevisiae* D5A (ATCC® Number 200062, incapable of fermenting xylose). This resulted in a net ethanol energy output (lignin energy excluded) of 4.55 GJ/ton wood (before distillation) when the pretreatment hydrolysate was detoxified using a resin column and the enzymatic and pretreatment hydrolysates were fermented separately (Zhu et al., 2010b). It was demonstrated that SPORL produces a lower amount of inhibitors than does dilute acid pretreatment under the same acid dosage for maximum sugar yield from the cellulose fraction (Shuai et al., 2010; Wang et al., 2009).

This study was conducted to evaluate the potential of combined fermentation of SPORL pretreatment hydrolysate and enzymatic hydrolysate from SPORL-pretreated solid substrate to maximize cellulosic ethanol production from lodgepole pine using an adapted strain *S. cerevisiae* Y5 without detoxification. The demonstrated tolerant inhibitor levels for the Y5 strain were 2, 0.5, and 10 g/L for furfural, HMF, and acetic acid, respectively (Li et al., 2009). Higher levels of tolerance were found based on our unpublished laboratory study. Combined fermentation of enzymatic hydrolysate with pretreatment hydrolysate can further reduce inhibitor concentrations through dilution in addition to supplementing glucose. Therefore, it is conceivable to use this combined fermentation approach to produce ethanol from SPORL-pretreated substrates without detoxification. This study is the first step towards maximizing ethanol yield while minimizing energy input from one of the most recalcitrant feedstocks, softwood lodgepole pine, without detoxification. No prior studies made such an attempt. Eliminating detoxification can avoid sugar losses and simplify the ethanol production process by using combined fermentation of the enzymatic and pretreatment hydrolysates. It can pave the way for future simultaneous enzymatic saccharification and combined fermentation with pretreatment hydrolysate. Mass and energy balance data obtained from such a study that accounts for both the cellulose and hemicellulose fractions can be used for more accurate economic analysis with commercial significance.

## 2. Experimental

The present experimental study from tree harvesting to fermentation was carried out according to the flow diagram shown in Fig. 1. The processes connected with dashed lines were not carried out in the present study.

### 2.1. Raw material and chemicals

A lodgepole (*Pinus contorta*) tree killed by mountain pine beetle (*Dendroctonus ponderosae*) (estimated infestation age of 4 years, abbreviated BD4) was harvested from the Canyon Lakes Ranger District of the Arapaho–Roosevelt National Forest, Colorado, located in the Colorado Front Range (east of the continental divide). Wood chips used were produced from breast height log of the tree. Detailed information about harvesting, transportation, and wood chipping are described elsewhere (Luo et al., in press).

Celluclast 1.5 L and Novozyme 188 (β-glucosidase) were generously provided by Novozymes North America (Franklinton, NC). Sodium acetate, sulfuric acid, sodium bisulfite, and Amberlite™ XAD-4 were used as received from Sigma-Aldrich (St. Louis, MO). All other chemicals, including culture media ingredients, were re-

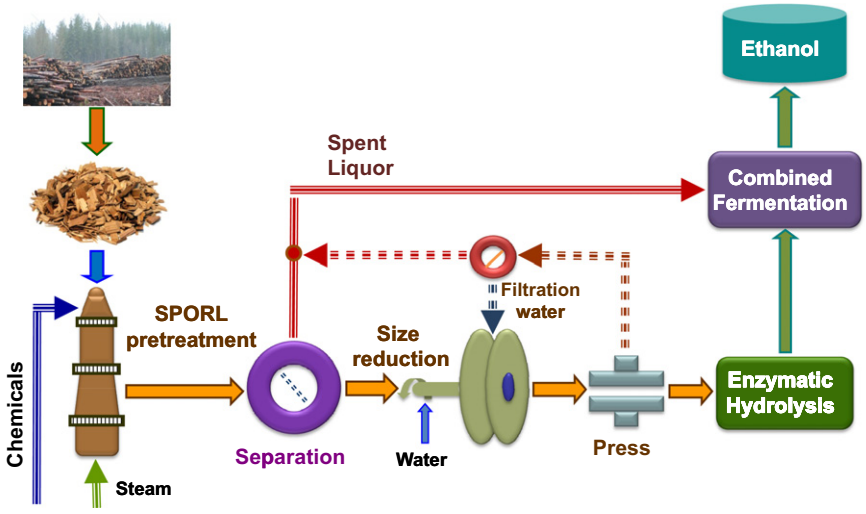


**Fig. 1.** Schematic process flow diagram of the SPORL process completed with ethanol fermentation. All steps, except those related to water recycling (dashed lines), were included.

ceived from Fisher Scientific (Hanover Park, IL). All chemicals were of analytical quality.

### 2.2. Microorganism and culture

*S. cerevisiae* Y5 (Strain preserved No. CGMCC2660, China General Microbiological Culture Collection Center) was obtained from Capital Normal University of Beijing, China. Details of the inhibitor-tolerance profiles of this yeast have been reported (Li et al., 2009). To prepare seed culture, 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 then transferred by loop to liquid YPD media supplemented with 30 g/L glucose in a flask. The *S. cerevisiae* Y5 seed was grown overnight at 30 °C with agitation at 200 rpm on a shaking bed until the biomass concentration reached approximately 2 g/L as monitored by optical density $OD_{600nm}$ measurements (Agilent 8453, UV–visible Spectroscopy system).

### 2.3. SPORL substrate production

The SPORL pretreatment was carried out according to the procedure described elsewhere using a 1-L wood pulping digester (Luo et al., in press; Zhu et al., 2010b). Wood chips of 150 g oven dry (od) weight, with a size about 6–38 and 3–8 mm in the thickness direction (Luo et al., in press), were directly subjected to digestion in a sodium bisulfite solution at 180 °C for 20 min. The pretreatment liquid-to-wood ratio (L/W) was 3 (v/w). The sodium bisulfite and sulfuric acid charges on od wood were 8% and 2.21% (both in w/w), respectively. These pretreatment conditions were based on our previous study of a live lodgepole pine tree (Zhu et al., 2010b). After the completion of pretreatment, the pretreated feedstock remained intact as wood chips and the wood chip solid yield was determined. The wood chip solids were then disk-milled to produce solid lignocellulosic substrate (Fig. 1). The energy consumption for disk milling was recorded as described elsewhere (Zhu et al., 2010b,c). The solid substrate yield was also determined for mass balance analysis. Several batch runs were required to generate enough material for the subsequent experiments. The solid and the liquid substrates were therefore combined into a large well-mixed batch of solid substrate and a large well-mixed batch of liquid substrate. The mixed solid and liquid substrates were stored in −16 °C for enzymatic hydrolysis and fermentation study. The pretreatment hydrolysate was first neutralized to pH 5 using lime before storage.

### 2.4. Pretreatment hydrolysate conditioning

To evaluate the potential of combined fermentation of enzymatic hydrolysate with pretreatment hydrolysate with and without detoxification, the pretreatment hydrolysates were conditioned before fermentation using three different methods: (1) simple neutralization to pH 5 using calcium hydroxide powder without a detoxification step, (2) detoxification by overliming, and (3) detoxification using XAD-4 resin. Overliming used slaked lime ($Ca(OH)_2$) powder, which was gradually added to the hydrolysate with constant stirring for 30 min until the pH of the hydrolysate reached 10. The overlimed hydrolysate was then centrifuged at 10,000*g* for 5 min to separate the precipitated calcium sulfate ($CaSO_4$). The supernatant of the overlimed hydrolysate was then mixed with sulfuric acid ($H_2SO_4$) to adjust it to pH 5. The detoxification of hydrolysate using XAD-4 resin was described in detail elsewhere (Weil et al., 2002; Zhu et al., 2010b), but briefly, Amberlite™ XAD-4 (15 g, Rohm and Haas, Phiadelphia, PA) was loaded into a 1.5 × 15 cm glass column and washed with 3× the column of water. The hydrolysate was then pumped onto the column at room temperature, and the eluate was collected once the hydrolysate began to exit the column as judged by color. Following absorption, the hydrolysate was neutralized with $Ca(OH)_2$ to pH 5. The three conditioned hydrolysates were all supplemented with yeast extract and peptone both at concentration of 0.5 g/L.

### 2.5. Separate enzymatic hydrolysis and combined fermentation of SPORL substrates

Enzymatic hydrolysate was first produced from SPORL-pretreated solid cellulosic substrate before combined fermentation with conditioned pretreatment hydrolysate commenced. Four identical hydrolysis treatments were conducted at a solid substrate loading of 10% (w/w) in 45 mL of sodium acetate buffer (pH 4.8, concentration 50 mM) using 5 g (od) solid substrate in 250-mL Erlenmeyer flasks. The substrate suspensions were incubated on a shaker (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 50 °C and 200 rpm. A mixture of Celluclast 1.5 L with an activity loading of approximately 15 FPU/g substrate and Novozyme 188 with an activity loading of approximately 22.5 IU/g substrate was used. After 12 h of enzymatic hydrolysis, 35 mL of one of the three conditioned pretreatment hydrolysates, either neutralized (undetoxified), overlimed, or XAD-4 detoxified, was added to each of the three enzymatic hydrolysates directly (without separating the enzymatic residue solids). The combined hydrolysates (i.e., 50 mL enzymatic + 35 mL pretreatment hydrolysate) were then inoculated by adding yeast *S. cerevisiae* Y5. The fourth enzymatic hydrolysate was combined with neutralized (undetoxified) pretreatment hydrolysate in a fed-batch mode according to the following schedule: 10, 10, 15 mL of the undetoxified pretreatment hydrolysate was added into 50 mL of enzymatic hydrolysate at 4, 24, and 48 h after inoculation, respectively. The starting yeast cell mass concentration was 2.0 g dry-cell wt/L. In an ideal industrial process, the solid and liquor streams will be combined and fermented together. To simulate this, it would be necessary for the 35 mL pretreatment liquor to be mixed with an enzymatic hydrolysate produced at 14% solids consistency based on solid substrate yield of 59.6% and L/W of 3. However, the enzymatic hydrolysate used in this study was produced at 10% solids consistency due to the difficulties in conducting 14% hydrolysis on shaking bed without a mechanical mixer. Therefore, the makeup of the combined hydrolysate (50 mL enzymatic + 35 mL pretreatment hydrolysate) represents overdosing of fermentation inhibitors. All fermentation experiments were carried out at 30 °C for 96 h. Samples were taken periodically and centrifuged at 10,000 × *g* for 5 min and were stored at −4 °C until analyzed for sugar and ethanol.

### 2.6. Analytical methods

The chemical compositions of the original and pretreated biomass were measured by the Analytical and Microscopy Laboratory of the Forest Products Laboratory as described elsewhere (Luo et al., in press; Zhu et al., 2010b). Ethanol analysis in the cellulosic substrate fermentation broth was carried out using a gas chromatograph (GC, model 7890, Agilent Technologies, Palo Alto, CA) through direct sample injection using an external standard for calibration. The sample was centrifuged at 15,000 × *g* for 5 min, and the supernatant was filtered through a filter (whatman 0.45 PDVF) before injection to the GC column. The chromatograph is equipped with a flame ionization detector and Agilent DB Wax column of 30 m with an ID 0.32 mm. A universal guard column was used to reduce column contamination. Sugar and inhibitor concentrations were measured using an HPLC equipped with an Econosphere™ C18 column (5-mm particle size, 250 mm × 4.6 mm, Alltech, Deerfield, IL) and a UV1000 ultraviolet detector (277 nm, Thermo Finnigan, San Jose, CA). Samples were run at ambient temperature and

eluted at 0.8 mL/min with a linear gradient of 50–100% acidified methanol (containing 0.25% acetic acid) run over 15 min. All analyses were carried out in duplicate at a minimum. The average data were reported. The standard deviations were calculated as measurement error.

## 3. Results and discussions

### 3.1. Saccharide recovery and the formation of fermentation inhibitors

Saccharide recovery and the formation of fermentation inhibitors can be used to assess the effectiveness of a pretreatment as they affect downstream processing. The SPORL pretreatment retained about 88% of the glucan on solid substrate and removed about 95% of the xylan and 97% of the mannan (Table 1), suggesting the pretreatment effectively fractionated the cellulose from the hemicelluloses. Separate enzymatic hydrolysis at 10% substrate solids indicated that over 90% of the retained glucan was converted to glucose after 48 h enzymatic hydrolysis at a cellulase dosage of 15 FPU/g substrate. Therefore, the SPORL pretreatment resulted in an enzymatic hydrolysis glucose yield (EHGY) of about 80% (88% × 90%) theoretical wood glucose. The EHGY of 80% is higher than the 75% reported from a beetle-killed lodgepole pine tree using $SO_2$-catalyzed steam explosion at 200 °C and $SO_2$ loading of 4% on wood (Ewanick et al., 2007). The tree used in the study was killed by beetles 3 years prior to harvesting in Central British Columbia, Canada, similar to the tree used in the present study, infested by the same pine beetle (*D. ponderosae*) for 4 years in the east of the continental divide of Colorado, United States.

A significant amount of the separated hemicelluloses in the pretreatment hydrolysate were not hydrolyzed to monomeric sugars despite the fact that hemicelluloses were efficiently fractionated from the cellulose by SPORL pretreatment. Mannose yield was about 51% (Table 1), lower than the values of about 65% achieved previously from lodgepole pine (Zhu et al., 2010b). Certainly, the pretreatment is not optimized with only one experimental condition. Furfural and HMF formations were about 16% and 9% calculated based on wood xylan and mannan content, respectively (Table 1). Furfural, HMF, and acetic acid concentrations in the pretreatment hydrolysate were 2.2, 2.7, and 5.3 g/L (Table 2), respectively. A previous study found that similar pretreatment hydrolysates from SPORL-pretreated lodgepole pine were fermentable with yeast *S. cerevisias* D5A (ATCC® Number 200062) only after detoxification using XAD-4 resin column absorption (Zhu et al., 2010b).

### 3.2. Fermentation of combined enzymatic and conditioned pretreatment hydrolysate with and without detoxification

The profiles of fermentation inhibitors and sugar in the conditioned pretreatment hydrolysate were determined before being combined with enzymatic hydrolysate for fermentation (Table 2). It was found that both detoxification methods (i.e., overliming and XAD-4 absorption) were effective in significantly removing inhibitors, especially furfural and HMF. The reductions of both furfural and HMF were about 40% and 60% by overliming and XAD-4 absorption, respectively. The reductions in acetic acid were 20% and 50% by overliming and XAD-4 absorption, respectively. Conflicting results on the effect of overliming on acetic acid reduction have been reported in the literature. No effect was suggested in an early study (Larsson et al., 1999). A reduction in acetic acid of 12% was reported in a recent study (Gupta et al., 2010). Another recent study found that overliming alleviated inhibition by acetic acid (Cheng et al., 2010). Simple neutralization (without detoxification) produced only minor reductions (~5%) in furfural, HMF, and acetic acid. The resultant initial concentrations of furfural and acetic acid, determined by multiplying the data in Table 2 by a dilution factor of 35/85, in the combined enzymatic and conditioned pretreatment hydrolysates were well below the demonstrated tolerance levels of the strain *S. cerevisias* Y5 of 2 and 10 g/L for furfural and acetic acid, respectively (Li et al., 2009). Initial HMF concentrations in the combined hydrolysates were higher than the demonstrated tolerance level of 0.5 g/L (Li et al., 2009) except for the sample using XAD-4 detoxification. Because the maximal tolerances for inhibitors of the stain Y5 were not thoroughly investigated, this

**Table 1**
Chemical composition of untreated wood chips and yields of key wood components in the solid substrate and pretreatment liquid hydrolysate. All data are in wt% of untreated wood unless otherwise indicated.

| | Untreated wood chips[a] | SPORL-pretreated solid substrate | SPORL pretreatment hydrolysate |
|---|---|---|---|
| |  |  |  |
| Glucan | 41.9 ± 0.6 | 36.7 ± 0.26/87.6%[b] | 2.7 ± 0.1/6.4%[b] |
| Xylan | 5.5 ± 0.5 | 0.3 ± 0.03/5.5%[b] | 2.2 ± 0.6/40%[b] |
| Mannan | 11.7 ± 0.3 | 0.3 ± 0.01/2.6%[b] | 6.0 ± 0.3/51.3%[b] |
| K. Lignin | 28.6 ± 0.2 | 21.3 ± 0.46/74.5%[b] | 7.3/(by balance) |
| Arabinan | 1.7 ± 0.2 | Nd | 0.6 ± 0.2/35.3%[b] |
| Galactan | 2.9 ± 0.4 | Nd | 1.4 ± 0.4/48.3%[b] |
| Furfural | | | 0.9/16.4%[d] |
| HMF | | | 1.1/9.4%[d] |
| Yield | 100 | 58.6/60.6[c] | 22.2 |

[a] The scale of the ruler is centimeter.
[b] The first number is sugar as polysaccharide; the second number after the back slash is saccharide yield, i.e., percentage of original sugar in wood.
[c] The first number is the sum; the second number is the measured solid substrate yield.
[d] The first number is furan as pentosan or hexosan; the second number after the back slash is furan as percentage of xylan and mannan in untreated wood.

**Table 2**
Effects of conditioning on pretreatment hydrolysate sugar and inhibitor profiles. All data are in g/L.

| Sample | Glucose | Xylose | Mannose | Furfural | HMF | Acetic acid |
|---|---|---|---|---|---|---|
| Initial liquor | 10.1 ± 0.40 | 8.3 ± 0.20 | 22.3 ± 0.70 | 2.24 ± 0.14 | 2.71 ± 0.15 | 5.26 ± 0.39 |
| Neutralized | 7.9 ± 0.79 | 6.3 ± 0.55 | 17.5 ± 2.02 | 2.17 ± 0.26 | 2.50 ± 0.02 | 5.09 ± 0.03 |
| Overlimed | 5.4 ± 0.36 | 3.9 ± 0.28 | 11.7 ± 0.89 | 1.36 ± 0.11 | 1.62 ± 0.02 | 4.18 ± 0.01 |
| XAD-4 absorbed | 4.6 ± 0.25 | 3.2 ± 0.18 | 9.9 ± 0.68 | 0.80 ± 0.05 | 0.91 ± 0.03 | 2.80 ± 0.05 |

study can provide some information about the performance of the strain Y5 using real pretreatment hydrolysates with and without detoxification. The detoxification also produced sugar losses. Compared to simple neutralization, glucose and mannose losses were 32% and 33%, 42% and 43%, for overliming and XAD-4 absorption, respectively. These numbers are higher than those reported in the literature (Larsson et al., 1999). The sugar losses will affect ethanol yield, as will be discussed later.

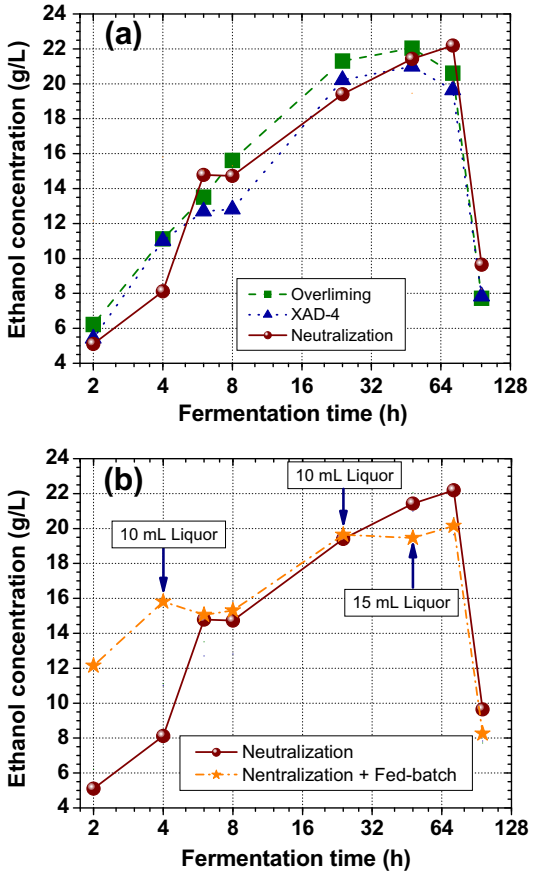


**Fig. 2.** Comparisons of time-dependent ethanol concentrations in fermentation broths: (a) neutralized (undetoxified), overlimed, and XAD-4-absorbed runs; (b) neutralized and neutralized + fed-batch run.

The time-dependent ethanol concentrations in the fermentation broth were examined to demonstrate the potential for combined fermentation of enzymatic and pretreatment hydrolysate without detoxification. It was found that the ethanol concentration profile for the run using neutralized (without detoxification) pretreatment hydrolysate is not much different from those obtained using overlimed and XAD-4-absorbed pretreatment hydrolysate (Fig. 2a). Detoxifications by overliming or XAD-4 absorption only improved ethanol productivity in the early stage from 2.0 to about 2.8 g/L/h (4 h, Table 3) but had negligible effects on the overall ethanol productivity (24 h, Table 3). However, the two detoxified runs had a slightly higher fermentation efficiency of about 10% over that of the neutralized run throughout the fermentation process. The higher fermentation efficiencies, however, did not translate into a higher ethanol concentration or yield due to losses of sugars through the detoxifications. Significant differences were observed when comparing the ethanol concentration profile of the neutralized run with that of the fed-batch run, especially in the initial period before pretreatment hydrolysate was added into the fed-batch run (Fig. 2b). Initial ethanol productivity in the first 4 h of the neutralized run was 2.0 g/L/h, only about half that of the fed-batch run before adding inhibitor-containing pretreatment hydrolysate (Table 3). This shows the negative effect of inhibitors on fermentation. However, the maximal ethanol concentrations of these two experiments are not much different.

The time-dependent sugar consumptions can verify the ethanol production results presented above. The two detoxified runs had slightly higher glucose consumption rates of about 7.7 g/L/h in the first 4 h compared with the corresponding 7.0 g/L/h for the neutralized run (Table 3, calculated using data in Fig. 3a). The glucose consumption rate for the fed-batch run in the first 4 h (without pretreatment hydrolysate or inhibitors) was about 13.0 g/L/h, or about two times faster than that of the neutralized run containing inhibitors. Without the addition of neutralized pretreatment hydrolysate that has low glucose concentration (Table 2) (i.e., without dilution) at the beginning of fermentation ($t = 0$) for the fed-batch run, the initial glucose concentration ($t = 0$) in the fed-batch run was higher than in the non-fed-batch runs (Fig. 3a). Detoxification significantly increased the mannose consumption rate (Table 3). There was a 2-h delay in mannose consumption for the neutralized run (Fig. 3b). Nevertheless, both glucose and mannose were almost consumed in about 6 h for all the experiments conducted (Fig. 3a and b). However, maximal ethanol concentration was not achieved until about 48 h or longer for all fermentation runs. The high ethanol productivity and sugar consumption rate achieved without detoxification can be explained

**Table 3**
Average ethanol productivity, rates of sugar consumption and furan metabolization in 4 (the first number) and 24 h (the number after back slash) of fermentation. All data are in g/L/h.

| Fermentation run | Neutralized | Overlimed | XAD-4 absorbed | Neutralized + fed-batch |
|---|---|---|---|---|
| Ethanol productivity | 2.03/0.81 | 2.78/0.89 | 2.75/0.84 | 3.95/0.82 |
| Glucose consumption | −7.02/−1.39 | −7.64/−1.35 | −7.75/−1.35 | −12.98/ |
| Mannose consumption | −0.28/ | −0.73/ | −0.92/ | |
| Furfural metabolization | −0.222/ | −0.135/ | −0.10/ | |
| HMF metabolization | −0.162/ | −0.135/ | −0.08/ | |

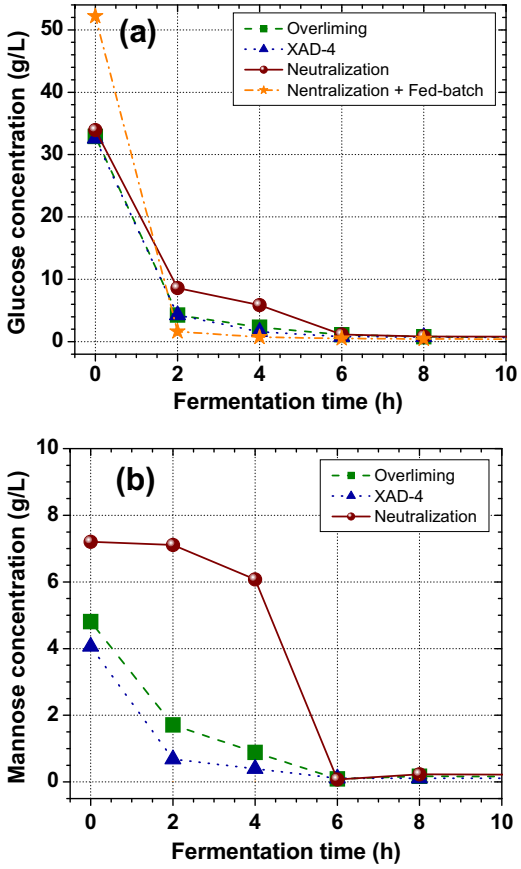


**Fig. 3.** Comparisons of time-dependent fermentable sugar concentrations in fermentation broths among different combined hydrolysate fermentation experiments: (a) glucose; (b) mannose.

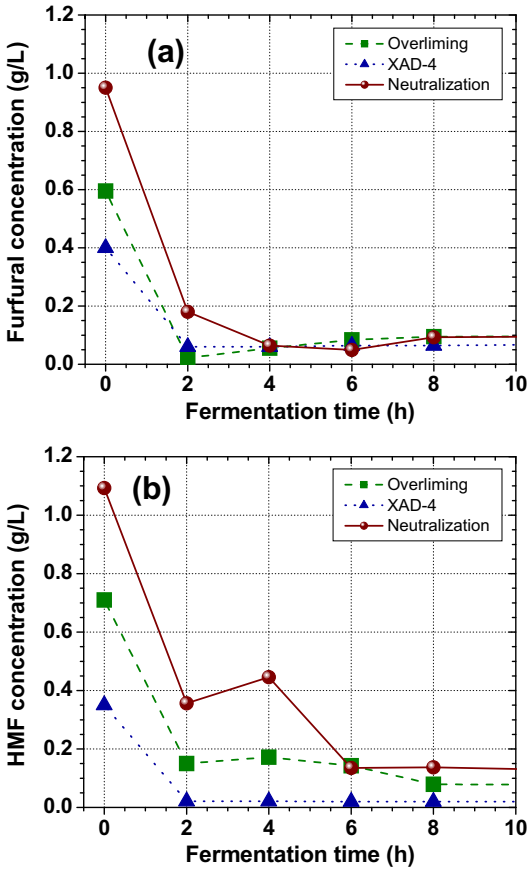


**Fig. 4.** Comparisons of time-dependent furan concentrations in fermentation broths among different combined hydrolysate fermentation experiments: (a) furfural; (b) HMF.

by two factors. First, the combination of enzymatic hydrolysate and pretreatment hydrolysate diluted the concentration of fermentation inhibitors. The dilution ratio between the enzymatic and pretreatment hydrolysate for the present study is about 1.4:1. This dilution ratio was used to calculate the initial concentrations of fermentable sugars (glucose, mannose) and inhibitors (furfural, HMF, acetic acid) in the combined hydrolysate. In addition, the combination of the two hydrolysates also supplements glucose, relative to the two types of detoxified hydrolysates. Second, the adapted yeast strain Y5 has a good ability to metabolize inhibitors. Both furfural and HMF were rapidly consumed in the first 6 h, as shown in Fig. 4a and b, even without detoxification. The furan metabolization rates of the neutralized run were significantly higher than the corresponding values of the two detoxified runs (Table 3), suggesting the Y5 strain can tolerate even higher furan concentrations than those presented in the combined hydrolysate. This explains why fed-batch did not improve fermentation efficiency over the neutralized run. Perhaps a fed-batch schedule should be redesigned especially when more pretreatment hydrolysate is added in future studies.

### 3.3. Preliminary evaluation of mass balance and net ethanol energy output

The SPORL pretreatment process data along with the results from the fermentation using the neutralized pretreatment hydrolysate (without detoxification) were used to determine process mass balance and net ethanol energy output (Fig. 5). The ethanol yield was 213 kg/ton wood or 270.4 L/ton wood, which is equivalent to 70.1% theoretical yield based on the wood glucan and mannan contents of 41.9% and 11.7% (Table 1), respectively. This ethanol yield is equivalent to that reported previously from live lodgepole pine trees utilizing completely separate fermentation of pretreatment hydrolysate after detoxification using XAD-4 resin (Zhu et al., 2010b). This ethanol yield is higher than the 242.7 L/ton corn stover, or 62.5% theoretical yield, obtained using AFEX-pretreated corn stover and a genetically modified *S. cerevisiae* 424A (LNH-ST) capable of fermenting xylose (Lau and Dale, 2009).

The following assumptions were made in determining process energy efficiencies and net energy output. Sub-processes shown in Fig. 1 with dashed lines were not carried out and therefore not included in the energy balance. Likewise, the enzymatic hydrolysis of solid substrates was not included in the preliminary energy balance because it was conducted using laboratory bench scale shakers as a batch process, which does not reflect industrial operations. Wood chipping energy was estimated at 50 W h/kg based on pulp and paper industrial experience. Thermal energy consumption for pretreatment was based on enthalpy of wood pulp at 25% consistency (L/W = 3) and 180 °C with the consideration of thermal energy recovery of 50%. Mechanical energy consumption for post-SPORL pretreatment wood chip size reduction was measured to be 212 W h/kg untreated wood. According to our previous description (Zhu and Pan, 2010), the pretreatment energy efficiency is defined as follows:

$$\eta_{\text{Pretreatment}} = \frac{\text{Total sugar recovery}}{\text{Total energy consumption for pretreatment}} \quad (1)$$

Similarly, the ethanol production energy efficiency or gain factor can be defined as net energy output divided by the total energy input (Zhu et al., 2010a,b):

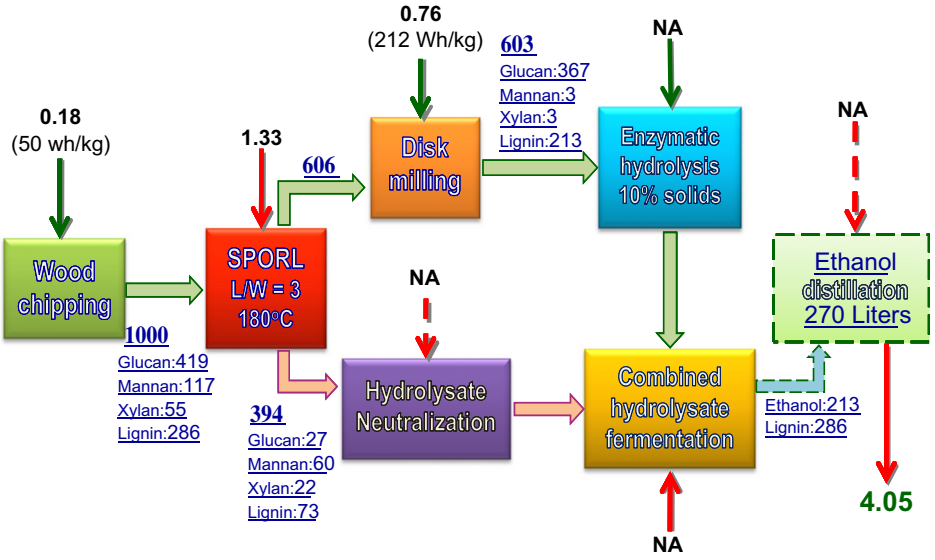


**Fig. 5.** Block diagram showing process mass and energy balance. Unless indicated, energy data are in GJ/ton wood and mass data are in kg.

$$\eta_{\text{Energy}} = \frac{\text{Net energy output}}{\text{Total energy input}} \quad (2)$$

For the present study, only ethanol energy was used in calculating net energy output. Total sugar recovery was the recovery of fermentable sugars, glucose and mannose, and was 85% based on the data in Table 1. Total energy consumption was 2.27 GJ/ton wood (Fig. 5). Therefore pretreatment and ethanol production energy efficiencies were 0.343 $GJ^{-1}$ and 178%, respectively. The net ethanol energy output was 4.05 GJ/ton wood (lignin energy not included), as shown Fig. 5. The figure provides a clear picture of component mass and process energy balance.

## 4. Conclusion

This study demonstrated the potential for robust ethanol production from softwood by SPORL pretreatment through fermenting the combined enzymatic and non-detoxified pretreatment hydrolysates using *S. cerevisiae* Y5. The adapted Y5 strain has good inhibitor tolerance and is capable of metabolizing furans while maintaining high ethanol productivity. Excellent ethanol yield was achieved without detailed pretreatment optimization. Future studies will focus on higher ethanol titer production using the combined fermentation approach demonstrated in this study to further evaluate the performance of the SPORL pretreatment and the Y5 strain to ferment hydrolysate with high inhibitor concentrations.

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