Efficient ethanol production from beetle-killed lodgepole pine using SPORL technology and Saccharomyces cerevisiae *without detoxification*

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ABSTRACT: This study applied Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (SPORL) to evaluate the potential of mountain pine beetle-killed lodgepole pine for ethanol production using conventional *Saccharomyces cerevisiae* without hydrolysate detoxification. The results indicate that the beetle-killed trees are more susceptible to SPORL pretreatment than live trees in addition to having enriched glucan and mannan content as reported in the literature. Ethanol yields of 200 and 250 L/metric ton wood were achieved from a live tree and a dead tree (four years after infestation) without process optimization. Ethanol yield of 220 L/metric ton of wood was obtained from a downed tree with more advanced decomposition, which is approximately 10% more than that from a corresponding live tree. Process mass and energy balance analyses suggest that net ethanol energy output (before distillation, lignin energy excluded) from the decomposing tree was approximately 3.2 GJ/metric ton wood, which is 23% more than that from a corresponding live tree. The study demonstrated the robustness of the SPORL process and the utility of beetle-killed trees for cellulosic ethanol production even after many years post mortality.

Application: Data from this study can help landowners and policy makers develop effective strategies for forest management and for use of beetle-killed lodgepole pine trees.

Mountain pine beetle *(Dendroctonus ponderosae)* infestation, although a natural disturbance, can result in extensive tree mortality and can significantly compromise public and private interests and land management objectives. Over 2.3 million acres of Colorado lodgepole pine (*Pinus contorta*) forests have been infested since 1996. Silvicultural treatments, such as thinning to remove the smaller trees in a stand and reduce competition among residual trees, can reduce the stand's susceptibility to infestation [1]. However, these smaller diameter trees removed during thinning treatments have limited value for structural materials, such as lumber. Large volume utilization of the thinnings of this material is critical to mitigate expensive thinning costs.

Cellulosic ethanol production from these low-value thinnings can be a potential pathway for large volume and value-added utilization. A previous study indicated that beetle-caused mortality enriched glucan content by as much as 3 percentage points (or 7.5%) [2]. Furthermore, the study found that beetle-killed trees were more susceptible to chemical pretreatment for effective enzymatic cellulose saccharification than live trees [2]. Similar results were also observed using beetle-killed lodgepole pine from British Columbia in Canada [3]. However lodgepole pine, a softwood, is very recalcitrant to biochemical conversion to ethanol. Few pretreatment technologies have proven to be effective in removing softwood recalcitrance for satisfactory enzymatic saccharification of cellulose [4]. Organosolv and acid-catalyzed steam explosion pretreatments have been applied to beetle-killed lodgepole pine [3, 5, 6]. These studies, however, did not investigate the effect of infestation age on the productivity of ethanol from wood. This parameter is critical to the utilization of these trees, because time between infestation and harvesting will vary from one location to another, depending on site conditions and land manager approaches. For these reasons, it is also imperative to determine the optimal time for harvesting after infestation.

The present study builds on our previous work on the evaluation of mountain beetle-killed lodgepole pines for sugar production [2]. The focus of the present study is on the effects of the length of time of beetle infestation on ethanol productivity using the SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses) process [7]. SPORL is a new forest biorefinery platform recently developed at the U.S. Forest Service, Forest Products Laboratory, in cooperation with the University of Wisconsin at Madison. SPORL is a robust and efficient pretreatment for removing the recalcitrance of woody biomass, including softwood species, for enzymatic saccharification with lower energy consumption when compared with acid-catalyzed steam explosion and organosolv processes [4, 7–9]. We have achieved an ethanol yield of approximately 270 L/ton of wood with a net energy

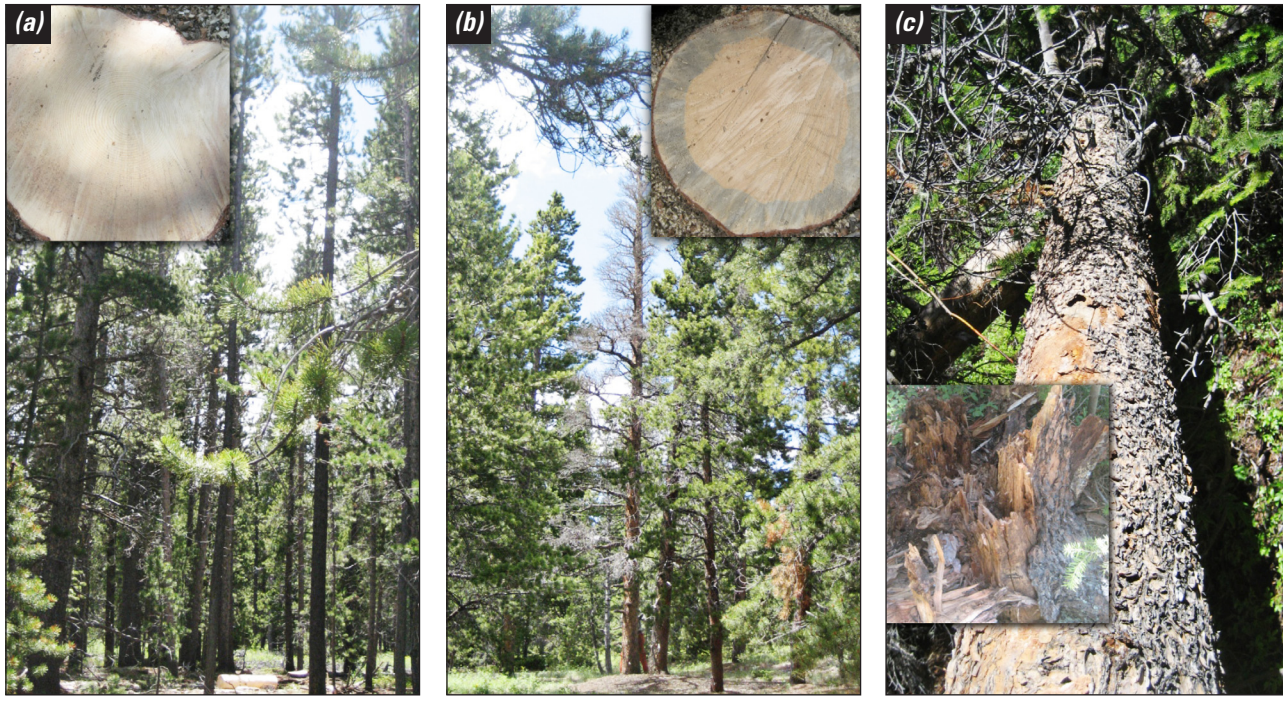
output over 4.0 GJ/ton of wood (before distillation) from lodgepole pine with and without detoxification in recent studies using SPORL [9, 10]. Proven commercial-scale facilities for implementing SPORL are available, such as digesters and disk refiners used in the pulp and paper industry, which significantly reduce technological and environmental risks compared with competing technologies, such as steam explosion, for which no commercial-scale facilities exist in the market. The data obtained from this study can provide information for landowners and policy makers to develop guidelines for identifying beetle-killed lodgepole pines that could be salvaged for utilization in the SPORL process. As bark beetle outbreaks are cyclical in nature and often occur across western conifer forests, there will likely be a continuous source of raw material.

EXPERIMENTAL

Materials

One living tree (as a control) and two mountain pine beetle-killed trees were harvested from each of the two selected sites at the Arapaho–Roosevelt National Forest, CO, USA, in July 2009: (1) the Canyon Lakes Ranger District, located in the Colorado Front Range (B), and (2) Fraser Experimental Forest, Sulphur Ranger District, located west of the U.S. continental divide (F). The Front Range (east side of the Rocky Mountains in Colorado, USA) has a dry climate and a relatively new insect outbreak of approximately four years, whereas the Fraser Experimental Forest has a wet climate (west side of Rocky Mountains in Colorado) where mountain pine beetle populations had been at outbreak levels for about nine years at the time of harvesting. All trees were approximately 100 years old with a diameter of 20–30 cm at breast height. Time since infestation of the dead trees varied and was determined by the rate of needle and twig loss [11]. The two living trees are identified as BL (**Fig. 1a**) or FL, and the beetle-killed trees are denoted as BD1, BD4 (Fig. 1b), FD5, and FDD8 (Fig. 1c), where the number indicates the years since infestation at harvest. FDD8 was a windfallen tree (about 8 years after beetle infestation), with about 75% of the bark missing and with weathered, exposed wood and moderately decayed sapwood (decay class 9 as described by Klutsch [12]) as evidenced from the image of the wood chips (**Fig. 2**). The specific tree conditions, along with GPS (UTM) coordinates of the trees, are as listed previously [2]. Dead trees also contained blue stain caused by a fungus (*Ophiostoma montium* or *O. clavigerum*) that is introduced into the tree by mountain pine beetles, as observed from the wood chips (Fig. 2). All trees were cut into 1.22-m long sections and debarked after falling, as described previously [2]. Each debarked log was wrapped in a plastic tarp to contain any potential beetles during shipping. The debarked logs were shipped to the U.S. Forest Service, Forest Products Laboratory, Madison, WI, USA, and then chipped and screened. The thickness of the accepted chips ranged from 3 to 8 mm (Fig. 2).

Celluclast 1.5 L and Novozyme 188 (β–glucosidase) were generously provided by Novozymes North America (Franklinton, NC, USA). The enzyme activities were 51.4 FPU/mL and 413 CBU/mL for Celluclast 1.5 L and Novozyme 188, respec-



1. Pictures of tree and tree stem cross section of selected live and beetle-killed lodgepole pine trees used in this study: (a) BL, (b) BD4, and (c) FDD8. Stem pictures from Luo et al. [2].

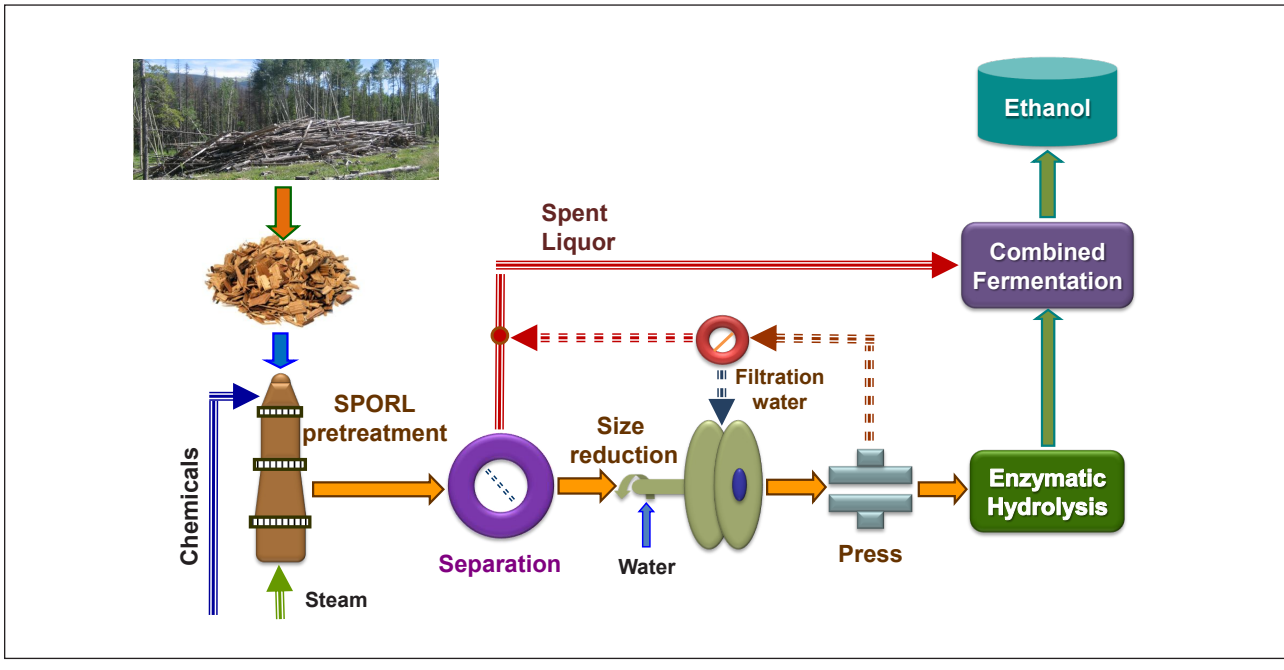
2. Wood chips from selected live and beetle-killed lodgepole pine trees used in this study: (a) BL, (b) BD4, and (c) FDD8 (from Luo et al. [2]).

tively, as determined through calibration [13]. Sodium acetate, sulfuric acid, and sodium bisulfite were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals, including culture media ingredients, were obtained from Fisher Scientific (Hanover Park, IL, USA). All chemicals were of analytical quality.

Yeast *Saccharomyces cerevisiae* Y5 (Strain preserved No.CGMCC2660, China General Microbiological Culture Collection Center) was supplied by Capital Normal University of Beijing, China. Details of the inhibitor-tolerance profiles of this yeast have been reported [14]. 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 of this strain was transferred from YPD-agar plates by loop to a fresh liquid YPD medium (10 g/L yeast extract, 20 g/L peptone, 30 g/L glucose) in a flask on a shaker (Thermo Fisher Scientific, Model 4450, Waltham, MA, USA). The *S. cerevisiae* Y5 seed culture was grown overnight at 35°C with agitation at 90 rpm on a shaking bed before harvesting. The harvested culture was centrifuged at 5,000 g for 5 min at 20°C to yield cell pellets after decanting the supernatant. Cell concentration (dry cell weight per liter) was calculated based on 600-nm absorbance. An aliquot was transferred to hydrolysate for an initial cell concentration of 2 dry cell weight per liter for fermentation.

SPORL pretreatment and size reduction of pretreated wood chips

All experiments were conducted according to the process flow diagram in **Figure 3**, similar to that described previously [10]. A detailed description of SPORL pretreatment and disk milling of pretreated wood chips for substrate produc-



3. Schematic process flow diagram of the experiments conducted. All steps except filtrate recycling (dashed lines) were included. Adapted from Zhu et al. [9].

tion can be found elsewhere [2, 9]. A dilute sodium bisulfite and sulfuric acid solution was used to mix with 150 g of o.d. wood chips at a liquor to wood ratio of 3:1 in a 1-L reactor. The charges of sulfuric acid and sodium bisulfite to o.d. wood were 2.2 and 8%, respectively. All pretreatments were conducted at 180°C for a period of 20 min. Three 1-L reactors were mounted inside a large rotating wood pulping digester as described elsewhere [7]. The reactor was heated externally using steam. All wood chips used in pretreatment were from a log (#1) at the breast height of the tree (1.4 m above ground). For each batch run, three different wood chip samples from different trees were separately placed in the three reactors and pretreated under the same conditions. Each 1-L reactor was cooled using tap water after being sealed following the completion of pretreatment. The pretreated wood chips were separated from the SPORL hydrolysate (spent liquor) using a simple screen. The pretreatment hydrolysate was poured into a plastic bottle and sealed and stored at 4°C until used for analysis and fermentation.

The pretreated wood chips were directly transferred to a laboratory 12-in. disk refiner (Andritz Sprout-Bauer Atmospheric Refiner; Springfield, OH, USA) for size-reduction under atmospheric conditions as described previously [2]. The size-reduced solids (substrate) were dewatered through pressing, using a canvas bag to a solid content of approximately 30%. The yield of solid (substrate) in the form of fibers or fiber bundles was then determined from the weight and moisture content of the collected substrate. The moisture content was determined gravimetrically by drying a sample of the collected solids in an oven at 105°C overnight. This solid substrate yield was used to convert the measured substrate glucan content and enzymatic hydrolysis glucose yield (EHGY) from substrate base to untreated wood base for process mass balance analysis. The electrical energy consumption for size-reduction by disk-refining was determined using a digital load monitor system (Ohio Semitronics; Hilliard, OH, model DLM-33-480-1PR) as previously described [15]. The determined energy was divided by the o.d. mass of the untreated wood chips to yield energy consumption for size reduction, in Wh/kg o.d. untreated wood.

Analytical methods

Chemical compositions of the wood and pretreated substrates were analyzed by the Analytical Chemistry and Microscopy Laboratory (ACML) of the U.S. Forest Service, Forest Products Laboratory. Details of sample preparation, analytical procedures, and instrumentation were described previously [2]. The saccharides and fermentation inhibitors in the pretreatment hydrolystaes (spent liquors) were analyzed using a Dionex high-performance liquid chromatographic (HPLC) system (ICS-3000) described elsewhere [2]. For rapid analysis, glucose in the fermentation broth was measured in duplicate using a commercial glucose analyzer (YSI 2700S, YSI, Yellow Springs, OH).

Ethanol analysis of the fermentation broths of cellulosic substrates and pretreatment hydrolysates was carried out using a gas chromatograph (Model 7890, Agilent Technologies, Palo Alto, CA, USA) through direct sample injection using an external standard for calibration. The chromatograph was equipped with a flame ionization detector (FID) and Agilent DB-Wax column of 30 m with an ID of 0.32 mm equipped with a universal guard column.

Separate enzymatic hydrolysis and fermentation

Separate enzymatic hydrolysis experiments of the six

Wood Log	Pretreatment Solid Yield (%)	Glucan Content in Solid (%)	Estimated Volume of Enzymatic Hydrolysate (mL)	Final Glucose Concentration in Enzymatic Hydrolysate (g/L)	Volume of SPORL Hydrolysate (mL)	Glucose Concentration in SPORL Hydrolysate (g/L)	Mannose Concentration in SPORL Hydrolysate (g/L)	Measured Glucose plus Mannose Concentration in the Combined Hydrolysate (g/L)
BL-1	61.9	58.6	55	41.2	24.2	8.0	17.0	37.7
BD1-1	61.4	61.3	55	48.8	24.4	8.7	19.5	44.3
BD4-1	62.2	61.4	55	49.5	24.1	9.0	21.8	45.5
FL-1	63.4	55.4	55	37.9	23.7	6.6	15.9	33.2
FD5-1	64.3	59.7	55	41.5	23.3	7.6	19.2	37.3
FDD-1	63.0	60.3	55	39.9	23.8	7.5	15.1	35.8

Liquor to wood ratio of 3:1 (V/w) in SPORL pretreatment; all enzymatic hydrolysates were produced from 5 g of solid substrate in 50-mL enzyme and buffer solution

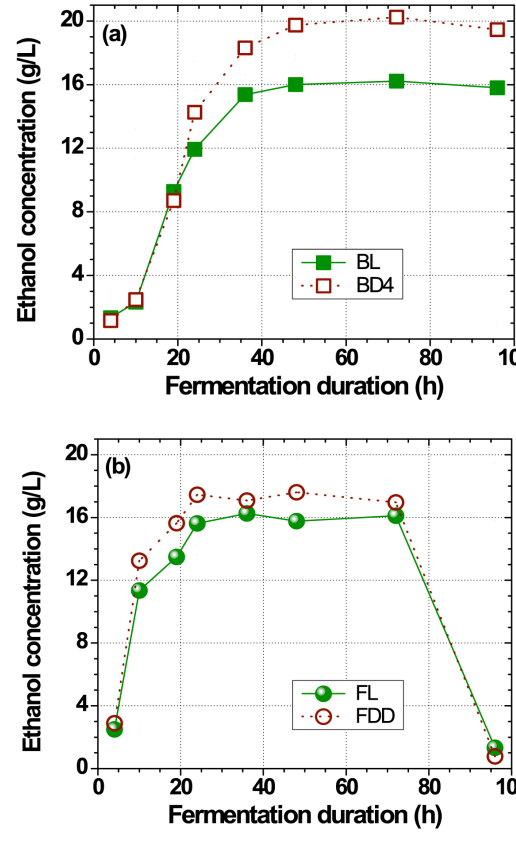
I. Makeup of the combined enzymatic hydrolysates and SPORL pretreatment hydrolystaes for fermentation of different tree samples.

Sample Label	Major Polysaccharides in Wood			Enzymatic Hydrolysate	SPORL Pretreatment Hydrolysate			Combined Hydrolysate
	Glucan	Xylan	Mannan	Glucose	Glucose	Xylose	Mannose	Glucose+Mannose
BL-1	398	68	101	281 / 64.1	24 / 5.5	31 / 40.1	51 / 45.9	369 / 66.7
BD1-1	403	53	116	330 / 74.4	26 / 5.9	25 / 41.5	59 / 45.8	432 / 74.9
BD4-1	419	55	117	339 / 73.5	27 / 5.9	25 / 40.0	65 / 50.8	447 / 75.2
FL-1	391	60	100	264 / 61.5	20 / 4.6	27 / 39.6	48 / 43.4	331 / 60.7
FD5-1	417	59	113	294 / 64.0	23 / 5.0	26 / 38.8	58 / 46.3	376 / 63.8
FDD-1	420	46	95	277 / 59.9	23 / 4.9	19 / 36.3	45 / 43.3	355 / 62.1

All data are in kg except the data after the slashes "/" that represent the percentage of theoretical yields. The data in the last column were based on measured sugar concentrations and volumes of the combined hydrolysate and pretreatment solid yields reported in Table I.

II. Fermentable sugar yields from enzymatic and SPORL pretreatment hydrolysates calculated in 1000 kg of wood.

pretreated solid substrates were conducted before performing combined fermentation with their respective SPORL pretreatment hydrolysate. Enzymatic hydrolysis was conducted using 5 g of substrate solids in 50 mL of sodium acetate buffer (concentration 50 mM, pH 4.8) on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50°C and 200 rpm. An enzyme mixture of Celluclast 1.5 L cellulase (25 FPU/g cellulose) and Novozyme 188 β-glucosidase (37.5 CBU/g cellulose) was used for hydrolysis. The final glucose concentration in the enzymatic hydrolysate was measured by the YSI glucose analyzer. For each tree sample, the volume of SPORL pretreatment hydrolysate used to combine with the enzymatic hydrolystate (approximately 55 mL) was determined based on the pretreatment liquid to wood ratio of 3:1 (V/w), the pretreatment solid substrate yield, and the weight of the solid substrate of 5 g used to produce the enzymatic hydrolysate (**Table I**). This makeup ensures that the ratio of pretreatment hydrolysate volume to solid substrate (5 g) is equal to the ratio of theoretical volume of pretreatment hydrolysate, i.e., 3 L/kg o.d. wood, to the experimental yield of solid substrate. Fermentations of the combined hydrolysates were carried out in 250-mL Erlenmeyer flasks using the shaker/incubator at 35°C and 90 rpm and buffered at pH 4.8. The open end of the flasks was wrapped with aluminum foil to prevent ethanol leak. No nutrients were added during fermentation. The initial yeast cell concentration was 2 g/L (wet basis). Samples from the fermentation broth were taken periodically for analysis. Reported results are the average of duplicate measurements.

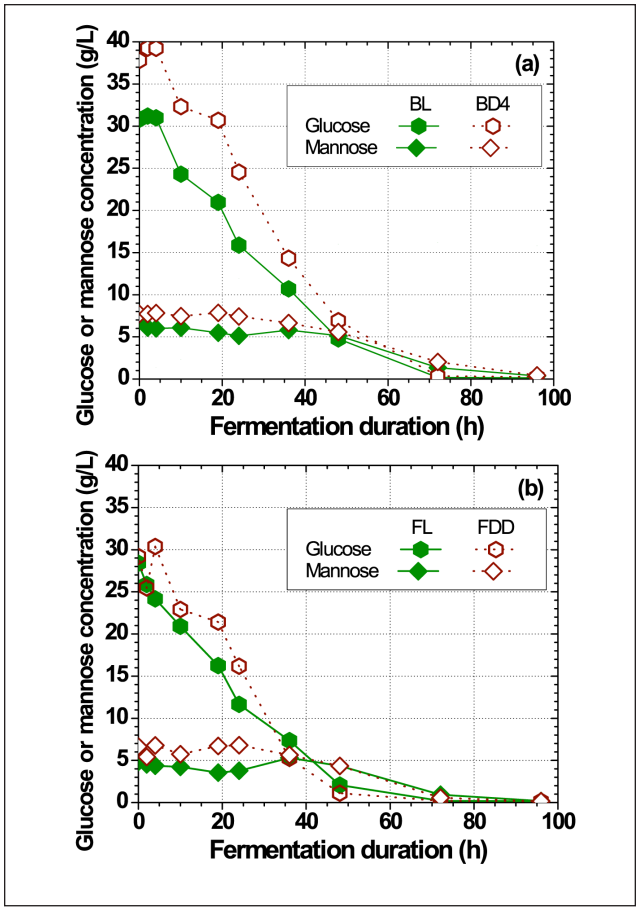


4. Effect of beetle infestation on time-dependent ethanol production in fermentation broths: (a) trees harvested from the Canyon Lakes Range District of the Arapaho–Roosevelt National Forest in the Colorado Front Range (B), and (b) trees from the Fraser Experimental Forest, Sulphur Range District of the Arapaho–Roosevelt National Forest in the Colorado Western Slope (F).

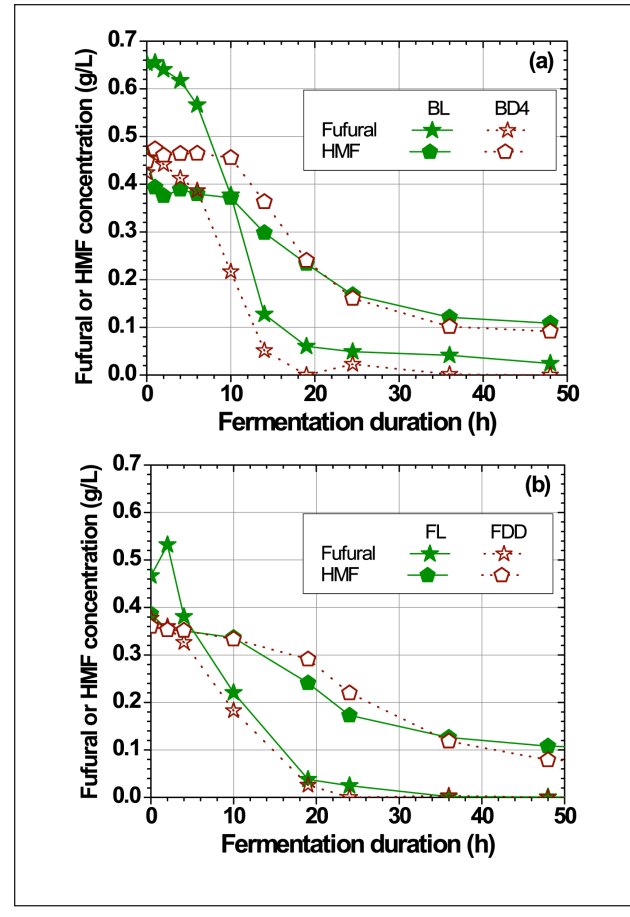
RESULTS AND DISCUSSION

Effects of beetle infestation on sugar yield

Beetle infestation enriched glucan and mannan content and reduced xylan content in lodgepole pine trees, as revealed in our previous study [2]. Furthermore, the enrichment increased with infestation age and can be captured by SPORL



5. Effect of beetle infestation on time-dependent glucose and mannose consumption in fermentation broths: (a) trees harvested from the Canyon Lakes Range District of the Arapaho–Roosevelt National Forest in the Colorado Front Range (B), and (b) trees from the Fraser Experimental Forest, Sulphur Range District of the Arapaho–Roosevelt National Forest in the Colorado Western Slope (F).



6. Effect of beetle infestation on time-dependent furan (fufural and hydroxymethylfurfual) metabolization in fermentation broths: (a) trees harvested from the Canyon Lakes Range District of the Arapaho–Roosevelt National Forest in the Colorado Front Range (B), and (b) trees from the Fraser Experimental Forest, Sulphur Range District of the Arapaho–Roosevelt National Forest in the Colorado Western Slope (F).

pretreatment followed by enzymatic hydrolysis. This is favorable for ethanol production, as fermenting hexose is much more efficient than fermenting xylose [16]. The major polysaccharide contents of the six trees measured in our previous study are duplicated in **Table II**. Although sugar yields of the six trees were reported in our previous study, the enzymatic hydrolysis was conducted at a very low solids consistency of 2% with enzyme loadings based on substrate solids. To provide subjective comparisons and be relevant to fermentation, enzymatic hydrolyses were conducted at 10% (w/V) with the same enzyme loading based on cellulose for all the six trees in the present study. The results indicate that enzymatic hydrolysis glucose yield (EHGY) increased with the increase in infestation age, except for tree FDD8 (Table II). It is possible that the pretreatment conditions may be too harsh for this sample, which can be seen from the slightly reduced glucan content retained on the solid substrate reported in our previous study [2]. However, the EHGY of FDD8 was still higher than that from its corresponding live tree FL. Similar results for mannose yield in the pretreatment hydrolysate were obtained (Table II). The low mannose yield from FDD8 was due simply to the reduced mannan content in the tree due to fungal decay. The fungal decay also reduced xylan content in the killed trees, which resulted in low xylose yield in the pretreatment hydrolysate (Table II). The measured glucose and mannose concentrations in the combined hydrolysates, together with the make-up volumes and pretreatment solid yields reported in Table I, were used to determine the final glucose and mannose yields. The results clearly indicated the increased yields of glucose and mannose from the beetle-killed trees (Table II). The hexose yield from BD4 was about 20% more than that from its corresponding live tree BL. Similarly, the yield form FD5 was about 14% more than that from its corresponding live tree, FL. The yield from FDD8 was about 7% more than that from FL. The maximum sugar yield of 75% theoretical wood glucan and mannan was achieved from tree BD4.

Sample Label	Wood Milling Energy [a]	Total Energy Input [a]	Ethanol Yield [b]	Ethanol Energy [a]	Net Energy Output	η_{energy} (%)
BL-1	0.59	2.10	202 / 56.2	4.72	2.62	125
BD1-1	0.61	2.12	245 / 65.6	5.73	3.61	170
BD4-1	0.65	2.34	252 / 65.4	5.91	3.57	152
FL-1	0.68	2.19	206 / 58.2	4.81	2.62	120
FD5-1	0.73	2.24	228 / 59.8	5.34	3.10	138
FDD-1	0.46	1.97	222 / 59.8	5.18	3.21	163

[a] in GJ/metric ton untreated wood.
[b] The first number is in L/of metric ton wood, and the second number is the percentage of theoretical ethanol yield based on glucan and mannon contents in wood (Table II).

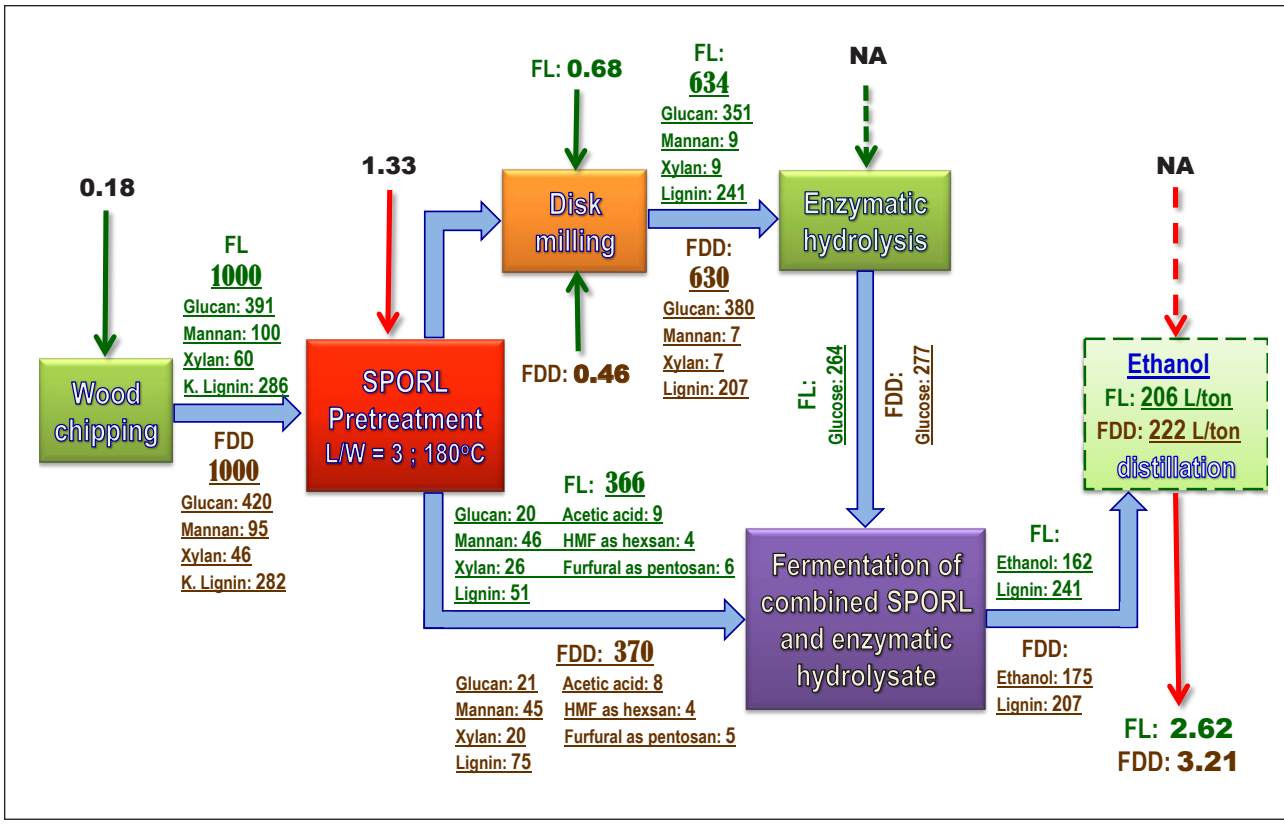
III. Comparisons of ethanol yields and ethanol production energy efficiencies from different trees.

Effects of beetle infestation on ethanol production

The combined hydrolysate has hexose (glucose+mannose) concentrations in a range of 33–45 g/L (Table I), with inhibitor concentrations of approximately 0.5 g/L for furfural and hydroxymethylfurfual (HMF) (to be discussed later) and of approximately 1.0 g/L for acetic acid (not shown). This suggests that the combined hydrolysate can be easily fermented using Y5 without detoxification based on our previous study [10]. The time-dependent ethanol concentrations in the fermentation broth were used to examine the effects of beetle infestation-induced fungal decay on ethanol production from infested trees. Only the results from BL and BD4 and FL and FDD8 were shown in this manuscript for clear presentation because the results from BD1 and FD5 fall between BL and BD4, and between FL and FDD, respectively. The results indicate that more ethanol was produced from the killed tree (BD4) than from the live tree (BL) (**Fig. 4a**), suggesting that beetle infestation-enriched glucan and mannan contents in wood can be captured after fermentation. The ethanol production rates in the first 20 h were approximately the same for both trees of 0.5 g/L/h. Similar results were also obtained for FDD8 and FL (Fig. 4b), i.e., more ethanol was produced from FDD8 than from FL, suggesting that the decaying tree (FDD8) was suitable for ethanol production. The final ethanol yields from the six trees were determined based on their terminal ethanol concentration in the fermentation broth. The results indicate that ethanol yields were higher from beetle-killed trees than from the corresponding live trees (**Table III**). Furthermore, the ethanol yield increased with infestation age. Ethanol yields from the two live trees (BL and FL) were about the same, slightly over 200 L/ton wood. The ethanol yield from BD1 and BD4 were 21% and 25%, respectively, more than that from the BL tree. Ethanol yield from the advanced decaying tree (FDD8) was about the same as that from FD5 with about 10% more than FL. The ethanol yield of 252 L/ton wood from BD4 was about 7% lower than the 270 L/ton wood reported in our previous studies [2, 10]. This is most likely because separate enzymatic hydrolysis and fermentation (SHF) of the SPORL solid substrate were carried out in this study, whereas quasi-simultaneous enzymatic hydrolysis and fermentation (q-SSF) was employed in both of the previous studies [2, 10].

The time-dependent sugar consumptions can directly verify the ethanol production results. For the same reason stated previously, the results from BD1 and FD5 were not shown for clear presentation. Glucose consumption was rapid for samples from both the live and beetle-killed trees (**Figs. 5a** and **5b**). The higher glucose concentrations in the fermentation broths of the beetle-killed samples (BD4 and FDD8) resulted in greater glucose consumption rates in the first 48 h, that is 0.69 and 0.60 g/L/h for BD4 and FDD8, respectively, and 0.55 and 0.5 for BL and FL, respectively. Mannose consumption was not noticeable in the first 40 h for all samples (Figs. 5a and 5b). Mannose consumption rates started to increase rapidly when glucose concentrations were reduced to the same level of mannose. Similar phenomena about mannose consumption were also observed in the non-detoxified sample using Y5 in our previous study [10]. Both glucose and mannose were almost completely consumed after about 80 h of fermentation in all experiments conducted.

Metabolization of furan (furfural and HMF) by the Y5 yeast was reported in our previous study [10] and was also observed in this study (**Figs. 6a** and **6b**). The metabolization of furfural was rapid and much faster than HMF. Furfural was metabolized in the first 20 h of fermentation for all the experiments conducted. However, metabolization of HMF was rather slow. HMF concentration was maintained at about 0.1 g/L after 50 h of fermentation for all experiments conducted (Figs. 6a and 6b). Complete metabolization of HMF was not achieved until the end of fermentation at about 96 h (not shown). The initial furfural concentrations in the fermentation broth from the live tree samples (BL, FL) were higher than those from the corresponding beetle-killed trees (BD4, FDD8)



7. A block diagram comparing process mass and energy balance between a tree (FDD8) decaying due to beetle infestation and a corresponding live tree (FL). Unless indicated, energy data (bold, italic font) are in GJ/ton wood and mass data (underlined) are in kg.

(Figs. 6a and 6b). This was likely because of the reduced xylan content in the beetle-killed trees resulting from fungal decay. The enriched glucan and mannan content in the beetle-killed trees did not, however, cause a correspondingly increase in HMF production (Fig. 6a). The reductions in furfural formation from beetle-killed trees did not produce tangible benefits for fermentation in the present study; however, the reductions will likely be beneficial for future high solids fermentation studies to obtain high ethanol titer.

Preliminary evaluation of net energy output in ethanol production

Preliminary evaluation of net energy output (before distillation) and energy efficiency for ethanol production was carried out using the ethanol data presented in Table III. The data in Table III (also Table II) are reported (calculated) in 1,000-kg base to provide an appropriate scale for industrial readers, although all experiments including pretreatment and disk milling were conducted on the order of 100 g (or mL). The ethanol production energy efficiency can be defined as [9, 10],

$$\eta_{Energy} = \frac{Net\ energy\ output}{Total\ energy\ input} \quad (1)$$

In Table III, only ethanol energy was considered in the energy output (energy from lignin was excluded). Only energy consumed for pretreatment was used to determine the total energy input. Mixing energy for enzymatic hydrolysis was ignored, as it was conducted in a shaking bed at a low consistency of about 10%. Wood milling energy was measured as described in the Experimental Section. Thermal energy for pretreatment was simply the increase in enthalpy of pulp suspension from thermal dynamic calculations with the consideration of 50% thermal energy recovery based on pulp mill experiences. The energy for wood log chipping was assumed at 50 Wh/kg based on pulp mill experience. Ethanol yield was calculated based on measured ethanol concentration at 72 h. Ethanol energy was determined based on the ethanol yield and ethanol high heating value (HHV) of 24 MJ/L. Energy efficiency was calculated using Eq. (1).

The results indicate that significantly more net energy was produced from the beetle-killed trees than from the live trees. For example, net energy output from trees BD1 and BD4 was 35% more than its corresponding live tree BL. Similarly, net energy output from FD5 and FDD8 was about 20% more than that from their live tree FL. This is of relevance, especially considering that the lignin content of the beetle-killed trees was about the same as that of the live trees based on our previous study [2]. This suggests that the high net energy output from ethanol for the beetle-killed trees was not offset by energy loss in lignin, the major component with high energy content. The determined ethanol production energy efficiencies were all over 100% (Table III); that

is, more net energy (ethanol only, before distillation) was produced than energy input.

A preliminary mass and energy balance evaluation for the more advanced decaying tree (FFD8) and its corresponding live tree (FL) were conducted based on the ethanol production data from this study and carbohydrate data from our previous study [2]. Using an estimated distillation energy of 5.6 GJ/ton ethanol [17], net energy output was about 1.6 and 2.2 GJ/ton wood from the live BL and dead tree FDD8, respectively. As **Figure 7** depicts, the process mass and energy balance for these two trees was the most comprehensive reported in cellulosic ethanol research. This type of process data can provide objective information for accurate process economic analysis.

CONCLUSIONS

This study applied a sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) technology to lodgepole pine to achieve efficient ethanol production using conventional *Saccharomyces cerevisiae* yeast without detoxification. When comparing the results from mountain pine beetle-killed trees to those from corresponding live trees harvested from the same site with equivalent age and growing conditions, we found that the beetle-killed trees are more susceptible to SPORL pretreatment and resulted in increased ethanol production. This is in addition to enrichments in glucan and mannan contents in the beetle-killed trees as reported in our previous study. Ethanol yields of 200–250 L/metric ton of wood, or about 56–66% theoretical value, were obtained from live and dead trees without process optimization using separate enzymatic hydrolysis and combined fermentation of the enzymatic and SPORL pretreatment hydrolysate without detoxification. This suggests that SPORL pretreatment for ethanol production from woody biomass, including softwood species, is robust. Ethanol yields from beetle-killed trees are as much as 25% more than from the corresponding live tree. As

ABOUT THE AUTHORS

The forest biorefinery potentially can help achieve energy independence and reduce climate change. Mountain pine beetle (*Dendroctonus ponderosae*) infestations, although a natural disturbance, can result in extensive tree mortality and can significantly compromise public and private interests and land management objectives.

More than 2.3 million acres of Colorado lodgepole pine (*Pinus contorta*) forests have been infested since 1996. These trees can be a viable feedstock for forest biorefineries. This study showed that Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (SPORL), a modified sulfite-based pulping process along with disk refining could effectively remove woody biomass recalcitrance for robust wood, including those of softwoods, for cellulose enzymatic saccharification in biorefinery applications. This study also demonstrates that wood severely decayed by fungi, such as the mountain pine beetle-killed and decayed lodgepole pine, is a very good feedstock for sugar and cellulosic biofuel production.

Our previous research was in the area of pulp and paper production. This research uses the knowledge developed in pulp and paper research and the infrastructure in the pulp and paper industry to develop technologies to forest biorefinering.

In this study, the strong recalciltrance of softwood species was the most difficult to remove for the forest biorefinery. We learned that the decayed lodgepople pine trees are enriched with glucan and mannan. Furthermore, decayed trees are more susceptible to pretreatment for enzymatic saccharification.

The SPORL process has potential future biorefinery applications. We are working on scale up of the SPORL process using woody biomass.

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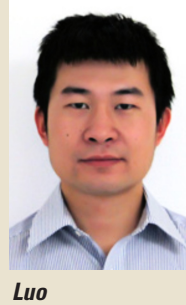
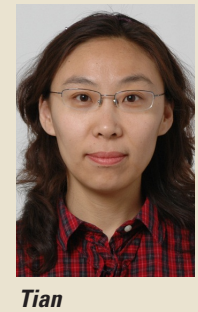
Zhu


Luo


Gleisner


Tian


Negrón


Horn

a result, net ethanol energy output (before distillation) from the beetle-killed tree was as much as 35% more than the corresponding live tree. Even a severely decayed tree produced 7% more ethanol yield and 20% more net energy output (before distillation) than the corresponding live tree. The process mass and energy balance data presented in this study can provide information for landowners, the forest products industry, and policy makers in determining the utility of beetle-killed trees when making decisions regarding utilization of killed trees. Because SPORL was developed based on mature wood pulping technology, it can be commercially implemented using existing capital equipment and infrastructure for building new mills or retrofitting existing pulp mills, which significantly reduces technological and environmental risks for commercialization. **TJ**

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