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On Polydispersity of Plant Biomass Recalcitrance and Its Effects on Pretreatment Optimization for Sugar Production

J. Y. Zhu · Steve P. Verrill · Hao Liu · Victoria L. Herian · Xuejun Pan · Donald L. Rockwood

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Abstract This paper discusses a property associated with plant biomass recalcitrance to enzyme and microbial deconstructions in sugar production from cellulose and hemicelluloses. The hemicelluloses are more readily hydrolyzed to sugars than is cellulose. As a result, optimization to maximize individual glucose and hemicellulose sugar recovery is not possible. This property is an inherent feature of plant biomass and is named polydispersity of plant biomass recalcitrance (PPBR) in this study. A set of pretreatment experiments using eucalyptus and sulfite pretreatment to overcome recalcitrance of lignocelluloses was conducted. The results were used to predict the conditions for individually maximizing enzymatic glucose and xylose yields. The predicted maximal yields were used to quantitatively illustrate the PPBR concept. The effect of PPBR on pretreatment optimization and strategies for maximal sugar recovery using two-stage pretreatment are discussed.

Keywords Biomass recalcitrance · Pretreatment · Optimization · Sugar recovery · Enzymatic hydrolysis/saccharification

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J. Y. Zhu · S. P. Verrill · V. L. Herian
USDA Forest Service, Forest Products Laboratory,
Madison, WI, USA

H. Liu
State Key Laboratory of Pulp and Paper Engineering,
South China University of Technology,
Guangzhou, China

J. Y. Zhu (✉) · H. Liu · X. Pan
Department of Biological Systems Engineering,
University of Wisconsin,
Madison, WI, USA
e-mail: jzhu@fs.fed.us

D. L. Rockwood
School of Forest Resources and Conservation,
University of Florida,
Gainesville, FL, USA

Introduction

Biomass feedstock recalcitrance to enzymatic and microbial deconstructions has long been recognized as a major barrier to efficient utilization of lignocellulose for sugar production. Pretreatment has been identified as a necessary step to remove the biomass recalcitrance [1, 2]. Most of the pretreatment research has been focused on developing various pretreatment processes for maximal cellulose enzymatic saccharification [3–5]. Process optimization has also been carried out to achieve maximal total sugar (hexose and pentose) yields through pretreatment (chemical hydrolysis) and subsequent enzymatic hydrolysis [6–9]. Pretreatment optimization is often carried out by conducting pretreatment experiments under a wide range of pretreatment conditions, such as temperature, time, and chemical dosage. Regression analyses are then performed to obtain equations that permit optimal pretreatment conditions [6, 8] to be calculated.

It is known that the optimal conditions for glucose yield and hemicellulosic sugar yield, specifically, the yield of xylose and mannose, may not be the same [6]. Hemicelluloses such as xylan and mannan can be easily hydrolyzed to monosaccharides and further degraded into other byproducts even by mild chemical pretreatments, whereas hydrolysis of cellulose is much more difficult and requires much harsher pretreatment. This difference in the response to pretreatment between cellulose and hemicelluloses is a property of plant

biomass. Although well known, this property of biomass recalcitrance has not been named, defined, or investigated [10, 11]. We propose to use the term "polydispersity of plant biomass recalcitrance" (PPBR) to describe this property. PPBR creates a multi-objective optimization problem in sugar production from biomass through pretreatment. A few studies have been conducted to explore the approach of two-stage pretreatment to alleviate the problem for maximizing total sugar recovery without providing the rational for the pretreatment conditions selected [10, 11]. The pretreatment optimization studies reported in the literature [6, 8] adopted a "plain aggregating approach" [12] in which the total sugar is simply the sum of glucose and the hemicellulosic sugars, mainly xylose and mannose, with a weighting factor of 1 for all hemicellulosic sugars, i.e., scalar aggregating. Because the achievable fermentation efficiencies of hemicellulosic sugars, especially five-carbon sugars, are often lower than the efficiency of glucose from the solid cellulosic fraction [13], weighting factors of less than 1 for hemicellulosic sugars may be warranted in optimizing the aggregating function—total sugar.

The objectives of this study are: (1) to explore ways to quantify the polydispersity of plant biomass recalcitrance and (2) to evaluate the effects of PPBR on pretreatment optimization especially when using a two-stage pretreatment approach. Eucalyptus wood was pretreated by sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) under various conditions. SPORL was recently developed by the present authors and shown to produce robust enzymatic saccharification from both softwoods [5, 14] and hardwoods [15, 16] and an excellent ethanol yield of 276 L/ton wood from lodgepole pine [17]. Regressions were then performed to determine the pretreatment conditions that maximize yields of xylose, glucose, and total sugar, respectively. The results were used to define the polydispersity of eucalyptus recalcitrance. The importance of the present study lies in the following two facts: (1) in addition to biomass productivity and cellulose recalcitrance, PPBR is a relevant predictor of the suitability of an energy crop for biochemical conversion to sugars. It can be immediately used for clone screening in the breeding of energy crops including short-rotation woody biomass through genetic modification and (2) a good understanding of PPBR can help to design effective pretreatment strategies, such as a two-stage process for maximal sugar recovery from lignocellulosic biomass.

Methods

Materials

The wood used in this study is representative of *Eucalyptus grandis* cultivars available for commercial planting in Florida. Specifically, a 12-year-old tree of cultivar G2 in central Florida was felled and cut into logs that were immediately shipped to the US Forest Service, Forest Products Laboratory (FPL), Madison, WI. The logs were debarked and chipped at the FPL. The wood chips were screened to remove particles greater than 38 mm or less than 6 mm in length to ensure smooth operation in disk milling for size reduction. The accepted chips had thicknesses ranging from 3 to 8 mm. The contents of the key chemical components of the wood chips were 28.5%, 41.8%, and 10.4% for Klason lignin, glucan, and xylan, respectively.

Celluclast 1.5 L and Novozyme 188 (β-glucosidase) were generously provided by Genencor (Palo Alto, CA) and Novozymes North America (Franklinton, NC), respectively. Sodium acetate, sulfuric acid, and sodium bisulfite were used as received from Sigma-Aldrich (St. Louis, MO). All other chemicals, including culture media ingredients, were received from Fisher Scientific (Hanover Park, IL). All chemicals were of analytical quality.

SPORL Pretreatment

The SPORL experiments were conducted according to the process flow diagram shown in Fig. 1. Wood chips of oven dry (od) weight 150 g and pretreatment solutions were placed in sealed stainless steel 1-L pressure vessels (manufactured in-house). These 1-L vessels were mounted inside a larger pressure vessel as described elsewhere [5] and heated externally via steam while rotating at the speed of 2 rpm. A total of 47 pretreatments were conducted at temperatures 160°C, 170°C, and 180°C (Table 1). Sulfuric acid concentrations ranged from 0 to 0.5% (*v*/*v*). The sodium bisulfite charge on the wood ranged from 0% to 8% in 2% increments. The pretreatment durations were 0, 15, or 30 min. The pretreatment liquor to od wood chip ratio (*L*/*W*) was fixed at 3 (*v*/*w*). (See the section titled "Quantification of PPBR Through Pretreatment Optimization" for further details.) It was expected that the wide range of pretreatment conditions would facilitate achieving maximal sugar recovery through process optimization. Following pretreatment, the residual solids remained as wood chips, which allowed an easy separation from the hydrolysate liquor using a simple screen. The pretreatment spent liquor, which mainly contains xylose or mannose, and glucose, was recovered and stored at 4°C until used for analysis.

Mechanical Wood Size Reduction

The pretreated wood chips were fed directly into a laboratory 8-in. disk refiner (Andritz Sprout-Bauer Pressurized Refiner, Springfield, OH, USA) to produce solid cellulosic substrate

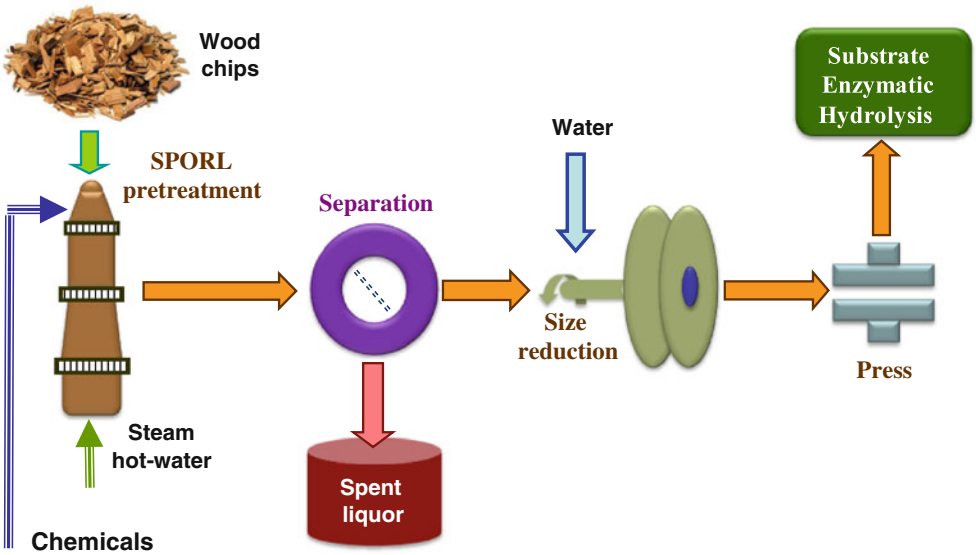

Fig. 1 A schematic process flow diagram of the sulfite pretreatment to overcome recalcitrance of lignocellulose SPORL process

(Fig. 1). The mechanical disk milling was carried out under ambient conditions with a disk plate gap of 0.25 mm. The disk mill was manually driven. About 1 L of water was added into the disk refiner with the pretreated wood chips to reduce the driving power required in the milling. The biomass collected from size reduction was dewatered using a Buchner funnel. The dewatered substrate was then washed five times using 500-mL water to obtain the substrate for enzymatic hydrolysis. Fast draining grade filter paper (Ahlstrom Grade 617, Mt Holly Springs, PA) was used for each dewatering step. The solid material from the filtration was stored for characterization.

Enzymatic Hydrolysis

Enzymatic hydrolysis experiments on the pretreated substrates were conducted to measure the enzymatic hydrolysis glucose yield (EHGY) in kilograms per ton of untreated wood. Enzymatic hydrolysis was conducted using commercial enzymes at 2% substrate solids (*w*/*v*) in 50-mL of sodium acetate buffer (pH 4.8, concentration 50 mM) on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) set at 50°C and 200 rpm. An enzyme mixture of Celluclast 1.5 L cellulase (7.5 FPU/g substrate) and Novozyme 188 β-glucosidase (11.25 IU/g substrate) was used for hydrolysis. Hydrolysate was sampled periodically for glucose concentration. Each data point is the average of two replicate analyses of the same sample.

Analytical Methods

The chemical compositions of the original eucalyptus and pretreated substrate were analyzed using improved high-performance anion exchange chromatography with pulsed amperometric detection [18]. Solid materials were Wiley milled (model #2, Arthur Thomas Co, Philadelphia, PA) using a 20-mesh outlet screen (~1 mm). The samples were first hydrolyzed using sulfuric acid in two stages. The hydrolysis conditions were an acid concentration of 72% (*v*/*v*) at 30°C and 3.6% (*v*/*v*) at 120°C for the first and second stages, respectively. The hydrolysis duration time was 1 h for both stages. The hydrolysate was then analyzed for carbohydrates using a Dionex HPLC system (ICS-3000, Dionex). The Klason lignin content was measured gravimetrically after washing and drying the solid residue from the acid hydrolysis.

The saccharides in the pretreatment hydrolysates (spent liquors) were analyzed as described elsewhere [19] using a Dionex HPLC system equipped with Carbopac™ PA1 guard and analytical columns at 20°C. Eluent was provided at a rate of 0.7 mL/min, according to the following schedule: 0–25 min, 100% water; 25.1–35 min, 30% water and 70% 0.1 M NaOH; 35.1–40 min, 100% water. To provide a stable baseline and detector sensitivity, 0.5 M NaOH at a rate of 0.3 mL/min was used as post-column eluent. Fermentation inhibitors generated in pretreatment, including acetic acid, formic acid, furfural, levulinic acid, and 5-hydroxylmethylfurural, were analyzed using the same system with a Supelcogel C-610 H column at temperature 30°C and UV detector at 210 nm. Eluent was 0.1% phosphoric acid at a rate of 0.7 mL/min. To determine the oligomeric saccharides, the pretreatment hydrolysates were further hydrolyzed by adding an equal volume amount of sulfuric acid of 6% (*v*/*v*) to make samples with sulfuric acid concentration of about 3% (*v*/*v*). The acid hydrolysis was conducted in an autoclave at 120°C for 60 min. All hydrolysate data reported were averages of triplicate measurements of the sample. For fast analysis, glucose in the enzymatic hydrolysate was measured in duplicate using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH).

Table 1 List of pretreatment conditions of all experiments and their corresponding measured yields of enzymatic hydrolysis (EH) glucose, pretreatment hydrolysate xylose, and total glucose and xylose

Sulfuric acid concentration (*v*/*v* %)	Sodium bisulfite (wt.% wood)	*T* (°C)	Pretreatment duration (min)	Percentage of theoretical yield (%)		
				EH glucose	Hydrolysate xylose	Total glucose+ xylose
0.1	4	160	0	3.64	0.20	2.96
0.2	4	160	0	3.82	2.87	3.63
0.3	4	160	0	8.50	0.91	6.99
0.1	4	160	15	34.52	8.05	29.26
0.2	4	160	15	54.65	29.26	49.61
0.3	4	160	15	61.43	37.44	56.66
0.1	4	160	30	46.33	36.22	44.32
0.2	4	160	30	64.10	72.28	65.73
0.3	4	160	30	67.62	68.31	67.76
0.1	4	170	0	23.49	3.23	19.46
0.2	4	170	0	56.90	17.19	49.01
0.3	4	170	0	67.95	23.70	59.16
0.1	4	170	15	58.95	43.71	55.92
0.2	4	170	15	76.69	67.85	74.93
0.3	4	170	15	83.47	79.69	82.72
0.1	4	170	30	70.16	69.67	70.06
0.2	4	170	30	83.18	75.03	81.56
0.3	4	170	30	87.29	73.51	84.55
0.1	4	180	0	66.33	22.63	57.65
0.2	4	180	0	76.29	44.27	69.93
0.3	4	180	0	79.75	46.21	73.09
0.1	4	180	15	81.37	60.58	77.24
0.2	4	180	15	88.47	68.03	84.41
0.3	4	180	15	91.42	67.70	86.71
0.1	4	180	30	84.81	56.35	79.16
0.2	4	180	30	94.07	58.61	87.03
0.3	4	180	30	87.96	60.71	82.55
0	0	180	30	46.91	18.31	41.23
0	2	180	30	60.31	42.74	56.82
0	4	180	30	64.20	47.38	60.86
0	6	180	30	80.90	41.16	73.01
0	8	180	30	78.33	38.00	70.32
0.2	0	180	30	77.01	45.54	70.76
0.2	2	180	30	89.98	57.32	83.49
0.2	4	180	30	92.45	56.55	85.31
0.2	6	180	30	91.83	51.47	83.81
0.2	8	180	30	91.03	49.61	82.8
0	0	180	30	47.48	16.62	41.35
0.1	0	180	30	79.87	47.96	73.53
0.2	0	180	30	81.27	46.48	74.36
0.3	0	180	30	81.30	25.14	70.14
0.5	0	180	30	59.96	4.15	48.87
0	4	180	30	64.22	51.35	62.82
0.1	4	180	30	84.97	56.89	83.12
0.2	4	180	30	88.23	57.98	86.19
0.3	4	180	30	83.74	55.29	83.10
0.5	4	180	30	79.83	26.03	77.13

Results

Observation of the Polydispersity of Plant Biomass Recalcitrance Through SPORL Pretreatment

PPBR has not been examined in a quantitative manner in the literature though it has been recognized. We will illustrate this property using the data obtained from eucalyptus in this study. For a given SPORL pretreatment temperature of 180°C and duration of 30 min, the sodium bisulfite charges for achieving "apparent" maximal enzymatic hydrolysis glucose yield (EHGY in 72 h) and xylose yield from pretreatment hydrolysate are not the same for the two sulfuric acid charges applied (Fig. 2). Both EHGY and xylose yield from pretreatment hydrolysate are presented as a percentage of sugar recovery, defined as the amount of sugar yield (EHGY or hydrolysate xylose yield) as a percentage of theoretical sugar, glucose, or xylose, respectively, in untreated wood. The error bars in Figs. 2 and 3 are the average standard deviations of the results from one triplicate and four replicate experiments (Table 1). We use the term "apparent" in the discussion in this section because the response curves shown in Figs. 2 and 3 are relatively flat and the optimal conditions may not be statistically distinguishable. The flat response curve is due to the low PPBR of the feedstock used, as will be discussed in the next section. At an acid charge on od wood of 1.1%, the apparent optimal sodium sulfite charges were about 4% and 2% to achieve maximal EH glucose and xylose recovery, respectively. When no acid is applied, the apparent optimal sodium sulfite charges were about 6% and 4%, respectively, to maximize EH glucose and xylose yields. The reductions of xylose yields at increased bisulfite charges (Fig. 2) were due to the increase in pH. Similar results were observed when examining the effect of acid charge on glucose and xylose recovery (Fig. 3). Pretreatments were again conducted at 180°C for 30 min. For dilute acid pretreatments (sodium bisulfite charge=0), the apparent optimal sulfuric acid charges that produced maximal glucose and xylose yields were about 1.1% and 0.55%, respectively. For SPORL pretreatments with bisulfite charge of 4%, the apparent optimal sulfuric acid charges were about 1.1% and 0.55%, respectively. More observations were made from experiments conducted by varying pretreatment temperature and duration. We found that the apparent optimal experimental conditions for achieving maximal EHGY were always different from those for achieving maximal xylose yield, which reflects the PPBR.

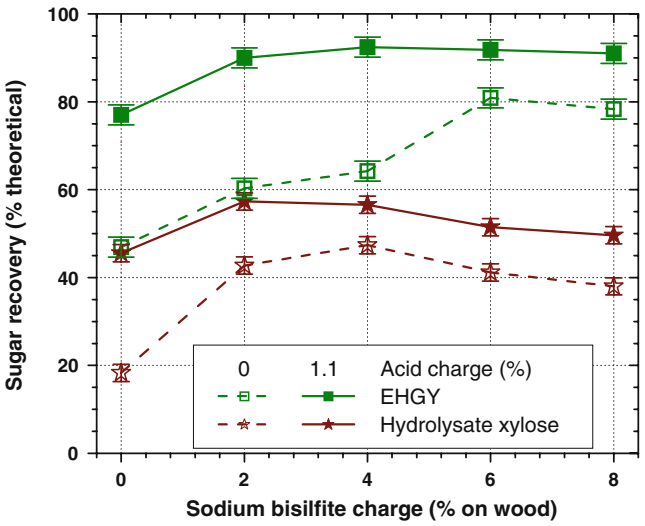

Fig. 2 Effect of sodium bisulfite charge on enzymatic hydrolysis glucose yield (*EHGY*) and yield of xylose in pretreatment hydrolysate. Pretreatments were conducted at 180°C for 30 min

The degree of this PPBR can also be observed from its effect on maximal sugar recovery as presented above. The flat response curves (Figs. 2 and 3) reflect that the PPBR of the eucalyptus studied is low. When SPORL pretreatments were conducted at 180°C for 30 min with acid charge of 1.1%, xylose yield from hydrolysate at sodium bisulfite charge 4% was very close to the apparent maximal value achieved at 2% (Fig. 2), the apparent optimal bisulfite charge for xylose. The apparent optimal bisulfite charge for EH glucose was 4% (Fig. 2). This means that using a bisulfite of 4% can achieve apparent maximal EH glucose yield and a very good xylose yield very close to apparent maximum. Consequently, the PPBR has a minor effect on maximizing sugar recovery. A similar observation can be made when examining the results obtained for pretreatments with varying sulfuric acid charges (Fig. 3). A flat sugar recovery curve of one sugar (EH glucose at sulfite charge=0, or xylose at sulfite charge=4) provides flexibility in choosing an acid charge to maximize recovery of the other sugar. These observations suggest that the eucalyptus wood studied has too low a degree of PPBR (to be quantified in the next section) to have a major impact on overall sugar recovery. Because PPBR is determined through sugar yield with a pretreatment, the observed effects of PPBR on sugar recovery are dependent on the pretreatment method applied.

Quantification of PPBR Through Pretreatment Optimization

PPBR is a property of plant biomass; therefore, it should be defined using plant biomass physical and chemical characteristics. However, such a definition is difficult given our current understanding of plant cell wall structure at the macromolecular or nano-scale. Moreover, PPBR can only be observed when biomass interacts with chemicals and enzymes through hydrolysis. Therefore, PPBR can be characterized and quantified using the sugar yield data

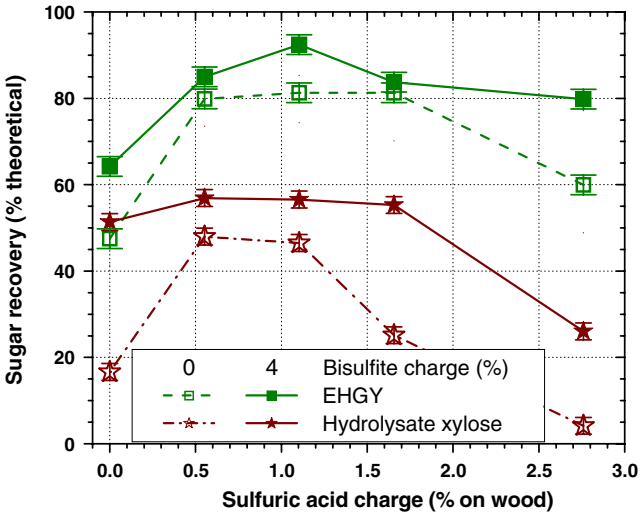

Fig. 3 Effect of sulfuric acid charge on enzymatic hydrolysis glucose yield (*EHGY*) and yield of xylose in pretreatment hydrolysate. Pretreatments were conducted at 180°C for 30 min

produced from a given pretreatment based on the discussion in the previous section. Specifically, we propose to use the following expression to quantify PPBR,

$$\mathrm{PPBR}_{1-2} = \frac{(\mathrm{Sugar})_1 - (\mathrm{Sugar1})_{1+2} + (\mathrm{Sugar2})_2 - (\mathrm{Sugar2})_{1+2}}{(\mathrm{Sugar1})_1 + (\mathrm{Sugar})_2} \tag{1}$$

where $(\mathrm{Sugar}\#i)_{\#j}$ represents the yield of sugar #*i* when optimized for maximal yield of sugar #*j*, and the sugars can be EH glucose from cellulose or xylose or mannose from hemicelluloses. In definition (1), sugars 1 and 2 are treated equally: i.e., a weighting factor of 1 is used in determining the aggregating function—(sugar 1+sugar 2). When only glucose and xylose are of interest, such as for most herbaceous biomass and hardwoods

$$\mathrm{PPBR}_{g-x} = \frac{(\mathrm{glucose})_g - (\mathrm{glucose})_{g+x} + (\mathrm{xylose})_x - (\mathrm{xylose})_{g+x}}{(\mathrm{glucose})_g + (\mathrm{xylose})_x}$$

$$= \frac{\Delta R_{\mathrm{glucose}} + \Delta R_{\mathrm{xylose}} \cdot XG}{(R_{\mathrm{glucose}})_g + (R_{\mathrm{xylose}})_x \cdot XG} \tag{2}$$

where $R_{\mathrm{glucose}} = \frac{0.9\cdot(\mathrm{glucose})}{\mathrm{glucan\ in\ wood}}$ and $R_{\mathrm{xylose}} = \frac{0.88\cdot(\mathrm{xylose})}{\mathrm{xylan\ in\ wood}}$ are glucose and xylose recovery (fraction of theoretical wood glucose or xylose), and $\Delta R_{\mathrm{glucose}}=(R_{\mathrm{glucose}})_g-(R_{\mathrm{glucose}})_{g+x}$ and $\Delta R_{\mathrm{xylose}}=(R_{\mathrm{xylose}})_x-(R_{\mathrm{xylose}})_{g+x}$ are the differences in glucose recovery and in xylose recovery between those obtained through optimizations for maximizing glucose or xylose and those for maximizing (glucose+xylose). $\Delta R_{\mathrm{glucose}}$ and $\Delta R_{\mathrm{xylose}}$ reflect the difference in the hydrolytic susceptibilities between cellulose and xylan of a given plant biomass to a particular pretreatment. *XG* is the ratio of plant biomass xylan (xylose) and glucan (glucose) content, i.e., $XG = \frac{45\ (\mathrm{xylan\ in\ wood})}{44\ (\mathrm{glucan\ in\ wood})}$. *XG* is a property of the plant biomass. A biomass with low xylan to glucan ratio tends to have a relatively low PPBR simply because xylan has a smaller share contributing to total sugar recovery. Obviously, PPBR of pure cellulose (*XG*=0) is zero. It can be shown that the maximum possible value for PPBR is 0.5 (or 50%).

Mannan is the major hemicellulose in softwoods, therefore, PPBR determination for softwoods can be written as follows according to Eq. 2,

$$\mathrm{PPBR}_{g-m} = \frac{(\mathrm{glucose})_g - (\mathrm{glucose})_{g+m} + (\mathrm{mannose})_m - (\mathrm{mannose})_{g+m}}{(\mathrm{glucose})_g + (\mathrm{mannose})_m}$$

$$= \frac{\Delta R_{\mathrm{glucose}} + \Delta R_{\mathrm{mannose}} \cdot MG}{(R_{\mathrm{glucose}})_g + (R_{\mathrm{mannose}})_m \cdot MG} \tag{3}$$

where $R_{\mathrm{mannose}} = \frac{0.90\cdot(\mathrm{mannose})}{\mathrm{mannan\ in\ wood}}$, $MG = \frac{\mathrm{mannan\ in\ wood}}{\mathrm{glucan\ in\ wood}}$. The PPBR of the eucalyptus wood used in this study can be quantified using the sugar yield data obtained from the SPORL pretreatment optimization experiments. The pretreatment conditions and corresponding yields, enzymatic hydrolysis glucose yield, xylose yield from pretreatment hydrolysate, and total yield of glucose (including glucose from pretreatment hydrolysate) and xylose, for these experiments are listed in Table 1. The first 27 observations constituted a 3×3×3 factorial experiment in acid, temperature, and time. Sodium was held constant. For the second 20 observations, temperature and time were held constant while acid and sodium were varied. Tests for systematic shifts in yield between the first 27 observations and the second 20 observations were not statistically significant, and the two subsets of data were combined in subsequent analyses. Given the overall design of the experiment, the sodium bisulfite charge main effect and the sodium/time and sodium/temperature interactions were confounded. The following quadratic model was fit to the data.

$$y = a_0 + a_1 \times x_1 + a_2 \times x_2 + a_3 \times x_3 + a_4 \times x_4 + a_{11} \times x_1^2 + a_{22} \times x_2^2 + a_{33} \times x_3^2 + a_{44} \times x_4^2 + a_{12} \times x_1 \times x_2 + a_{13} \times x_1 \times x_3 + a_{14} \times x_1 \times x_4 + a_{23} \times x_2 \times x_3 \tag{3}$$

where

$$x_1 \equiv \frac{\text{sulfuric acid concentration } \left(\frac{v}{v}\%\right) - 0.2}{0.1} \tag{4a}$$

$$x_2 \equiv \frac{\text{temperature} - 170}{10} \tag{4b}$$

$$x_3 \equiv \frac{\text{time} - 15}{15} \tag{4c}$$

$$x_4 \equiv \frac{\text{sodium bisulfite charge} - 4}{4} \quad (4d)$$

and sugar yield or recovery y is expressed as the percentage of theoretical sugar in untreated wood based on carbohydrate content. The critical points for the fitted quadratic models were found by setting their partial derivatives to zero. We verified that the critical points corresponded to maxima by checking that the matrices of second-order partial derivatives were negative. The estimated coefficients for the quadratic fits are reported in Table 2. The critical points are reported in Table 3. We note that tests for lack of fit of the quadratic models to the yield data are statistically significant, and that uncertainties are associated with the values of the estimated optimal treatment conditions. However, the present results are adequate for the purpose of illustrating the PPBR concept. In Table 3 we also report percentage recoveries under varying conditions. For example, in column 6 of Table 3, we report the predicted percent recoveries (based on the quadratic fits) of glucose under conditions that are optimal for glucose recovery, xylose recovery, and total recovery (101.9, 87.3, and 100.1, respectively).

Given the data in Table 3, $(R_{\text{glucose}})_g$=101.9, $(R_{\text{glucose}})_{g+x}$=100.1, $(R_{\text{xylcose}})_x$=72.9, and $(R_{\text{xylcose}})_{g+x}$=59.1, and eucalyptus wood glucan and xylan contents 0.418 and 0.104, respectively, we can illustrate the calculation of PPBR of eucalyptus using Eq. 2.

$$\Delta R_{\text{glucose}} = \left(R_{\text{glucose}}\right)_g - \left(R_{\text{glucose}}\right)_{g+x} = 101.9 - 100.1 = 1.8$$

$$\Delta R_{\text{xylose}} = \left(R_{\text{xylose}}\right)_g - \left(R_{\text{xylose}}\right)_{g+x} = 72.9 - 59.1 = 13.8$$

$$XG = \frac{45(\text{xylan in wood})}{44(\text{glucan in wood})} = \frac{45 \times 0.104}{44 \times 0.418} = 0.2545$$

Substituting these values into Eq. 2, we have $PPBR_{g\text{-}x}$=4.5% or 0.045. Based on the discussion in the previous section, a PPBR of 0.045 is considered to be low. If a weighting factor less than 1 is used for xylose in determining the aggregating function—total sugar of glucose and xylose, the optimal conditions for total sugar recovery will be even closer to those for optimizing glucose recovery and a smaller PPBR can result.

Two more data sets [3, 6] from Professor Saddler's group at the University of British Columbia, Canada, were also used in this study to demonstrate the PPBR concept. These two sets were selected because of their completeness and accessibility (one of the co-authors (Pan) of the present study is the major author of these two literature works). Furthermore, few literature studies provided such complete data sets for the calculation of PPBR using Eqs. 2 or 3. The organosolv pretreatment adopted in these two studies did not produce theoretically maximal yields of glucose and xylose or mannose in the range studied. That is, the quadratic fits had no maxima. Therefore, "largest observed value" maxima were used in the following calculations. The sugar recovery data along with the calculated PPBR using Eqs. 2 and 3 are listed in Table 4. The results suggest that lodgepole pine has a stronger PPBR than poplar. This is probably due to the strong recalcitrance of the softwood cellulose. As will be discussed below, the multivariable organosolv and SPORL pretreatments (both have four variables) may be effective in removing PPBR, which resulted in low PPBR for the three examples presented above.

Table 2 Coefficient estimates from three regression fits to Eq. 3

	EH glucose		Hydrolysate xylose		Total glucose and xylose	
Coefficients	Estimate	p value	Estimate	p value	Estimate	p value
a_0	76.78	0.0001	60.88	0.0001	73.23	0.0001
a_1	8.98	0.0001	7.24	0.0006	8.83	0.0001
a_2	22.83	0.0001	11.97	0.0001	20.99	0.0001
a_3	16.88	0.0001	21.97	0.0001	18.21	0.0001
a_4	8.26	0.0038	4.07	0.3057	8.23	0.0018
a_{11}	−3.56	0.0001	−4.23	0.0001	−3.42	0.0001
a_{22}	−6.45	0.0140	−10.03	0.0098	−6.84	0.0049
a_{33}	−10.26	0.0002	−11.49	0.0035	−10.19	0.0001
a_{44}	−3.74	0.2564	−17.67	0.0007	−7.84	0.0120
a_{12}	−2.54	0.0938	−4.53	0.0447	−2.65	0.0572
a_{13}	−2.10	0.1629	−2.64	0.2325	−1.93	0.1621
a_{23}	−2.10	0.1985	−0.48	0.8399	−0.99	0.5027
a_{14}	−9.38	0.0001	−10.36	0.0001	−9.10	0.0001

Table 3 Optimal pretreatment conditions derived from regression fits to sugar recovery data from the 47 experiments

Sugar used for optimization	Optimal pretreatment conditions				Predicted sugar recovery (%)		
	Sulfuric acid concentration (*v*/*v* %)	Temperature (°C)	Duration time (min)	Sodium bisulfite charge (wt.% wood)	EH glucose	Hydrolysate xylose	Total glucose and xylose
EH glucose	0.236	187	15.2	8.02	101.9	37.6	90.3
Hydrolysate xylose	0.257	170	28.3	4.43	87.3	72.9	85.0
Total glucose and xylose	0.267	182	19.3	5.93	100.1	59.1	93.5

Discussions

Relation Between Sugar Yield and PPBR, Productivity, and Recalcitrance

PPBR, along with productivity, and recalcitrance, should be used for clone screening in the breeding of energy crops including short-rotation woody biomass through genetic modifications. This can be seen from the following mathematical expression relating sugar yield to these three factors:

$$Y = P \cdot y = P \cdot \left[(\text{glucose})_{\text{TS}} + (\text{hemisugar})_{\text{TS}}\right]$$

$$= P \cdot G\frac{(\text{glucose})_g}{G} \cdot \frac{(\text{glucose})_{\text{TS}}}{(\text{glucose})_g} + H\frac{(\text{hemisugar})_h}{H} \cdot \frac{(\text{hemisugar})_{\text{TS}}}{(\text{hemisugar})_h} \quad (5)$$

where Y is total maximal sugar yield form a given area of land, y is maximal sugar yield from a unit mass of plant biomass, and P is biomass productivity. Subscript TS indicates conditions that lead to maximal total sugar yield. Subscripts g and h indicate conditions that lead to maximal glucose and hemicellulosic sugar recovery, respectively. G and H are glucan (cellulose) and hemicellulose content. In Eq. 5, $\frac{(\text{glucose})_g}{G}$ and $\frac{(\text{hemisugar})_h}{H}$ are related to the recalcitrance of plant biomass. $\frac{(\text{glucose})_{\text{TS}}}{(\text{glucose})_g}$ and $\frac{(\text{hemisugar})_{\text{TS}}}{(\text{hemisugar})_h}$ are related to PPBR. The smaller these two factors, the larger the PPBR as can be seen from Eq. 1. Equation 5 explains why PPBR should be a factor in clone screening.

Effect of PPBR on Pretreatment Optimization

The effect of PPBR on pretreatment optimization is dependent on the pretreatment method used. For example, the high pH SPORL pretreatment (sulfuric acid charge=0) has a relatively narrower range of sodium sulfite charge within which the recoveries of both EH glucose and hydrolysate xylose are flatter (relatively constant) than the low pH SPORL pretreatment (acid charge on od wood=1.1%; Fig. 2). Similarly, the dilute acid pretreatment (sulfite charge=0) has a narrower range of sulfuric acid charge within which the variations in EH glucose and xylose recoveries are relatively small than does the SPORL pretreatment (sulfite charge on od wood=4; Fig. 3). This low sensitivity of sugar recovery to a particular pretreatment variable can provide flexibility for process optimization. For example, optimization can be focused on some other objective targets such as low inhibitor formation to facilitate fermentation.

One way to respond to PPBR is to design a two-stage process. The pretreatment conditions for maximizing EH glucose, hydrolysate xylose, and total glucose and xylose (Table 1) can be schematically shown in a 2-D plot (Fig. 4). The conditions that maximize total glucose and xylose using single-stage pretreatment are listed in row 3 of Table 3. The xylose yield was 59%, or about 14 percentage points lower than the maximal achievable value of 73%

Table 4 Sugar recovery data from references [3] and [6] for the calculation of the PPBR of the feedstock examined

Wood	Sugar recoveries/run no.[a]				XG or MG	PPBR (%)
	$(\text{Glucose})_g$	$(\text{Xylose})_x$ or $(\text{mannose})_m$	$(\text{Glucose})_{g+x}$ or $(\text{glucose})_{g+m}$	$(\text{xylose})_{g+x}$ or $(\text{mannose})_{g+m}$		
Poplar [3]	84.8/12	58.2/14	81.0/17	52.5/17	0.415	5.7
Lodgepole pine [6]	72.7/17	62.5/5	72.7/17	41.0/17	0.259	6.3

[a] The first number is the largest observed value of sugar recovery in % of theoretical yield and the number after the slash is the corresponding run number in Table 1 in [3] or Table 2 in [6]

(Table 3), despite this, a total glucose and xylose yield of 93% theoretical was achieved. Because the conditions that maximize EH glucose recovery are more severe than those that maximize xylose recovery, a two-stage approach can be used to optimize both xylose and glucose recovery. Based on the low sensitivity of sugar recovery to acid and sulfite charge, we can set the acid and bisulfite charges of 0.25% and 6%, respectively, close to the average of those values for maximizing EH glucose and hydrolysate xylose (Table 1). Then we can focus on the other part of the solution, pretreatment temperature and duration time, in designing a two-stage pretreatment process. A logical design of the first stage in the process would be a low temperature stage with pretreatment conditions close to those for maximizing xylose recovery, i.e., a low pretreatment temperature of about 170°C and a long pretreatment duration of about 28 min. The second stage in the process would be a short stage at a high temperature. Experiments need to be carried out to optimize the second stage. The conditions are expected to be milder than those for optimizing EH glucose as the wood chips have been pretreated.

It might be possible to achieve near complete enzymatic saccharification of the cellulose in the resultant substrate from the second stage. However, the amount of glucan content retained on the final solid substrate may be lower than that achieved while maximizing EH glucose using a one-stage pretreatment because of glucan degradation in both stages. As a result, it is not certain if a two-stage process will achieve more EH glucose than that achieved through a single-stage optimization, especially for feedstock with low PPBR such as the eucalyptus used in this study. A two-stage process might also require extra energy input. A two-stage pretreatment could be carried out without using the step function temperature profile. A linear temperature profile ramping to merge the two stages (Fig. 4) may be an effective approach to maximizing total glucose and xylose or mannose yield, which was recently demonstrated using steam explosion pretreatment on a softwood [20].

PPBR Determination Under Special Scenarios

The above definition of PPBR also reflects the susceptibility of plant biomass to a particular pretreatment in addition to the inherent nature of plant biomass recalcitrance. The method presented above assumes that a maximum can be found for EH glucose and a hemicellulose sugar, e.g., xylose, through process optimization. However, it is possible that certain pretreatment methods may not produce a maximum in sugar recovery, in particular when many process variables are used such as in the organosolv pretreatment [3, 6] discussed above. A couple of scenarios can occur: (1) constant sugar recovery for both EH glucose and a hemicellulosic sugar, xylose or mannose, in a shared range or in respectively different ranges of pretreatment conditions and (2) multiple local sugar recovery maxima under different pretreatment conditions. The first scenario suggests that the plant biomass under study most likely has a very low PPBR. If the ranges of the pretreatment conditions that produced constant sugar recovery for EH glucose and a hemicellulosic sugar, xylose or mannose, are the same or overlap, then PPBR=0. If the ranges of the pretreatment conditions do not overlap, this scenario still has more flexibility in process optimization. The second scenario can occur when pretreatment processes have several process variables that all have strong effects on sugar recovery. A practical solution in this case is to decrease the pretreatment conditions to a small range to search for local maxima for the determination of PPBR using Eq. 2, as we demonstrated in this study using organosolv pretreatment data from the literature [3, 6]. Theoretically speaking, this scenario is an artifact of the pretreatment method, rather than a property of biomass recalcitrance. Therefore, a simple pretreatment such as a hot water process with only two variables, pretreatment temperature and duration, is suggested to reduce the number of pretreatment variables to avoid this difficulty in determining PPBR using the method presented in this study. The first scenario can also be an artifact of the pretreatment process due to the nature of plant biomass with a low PPBR. Using a range of mild pretreatment conditions and a simple pretreatment method such as hot water or dilute acid should reduce the probability of the problem of multiple local maxima in sugar recovery.

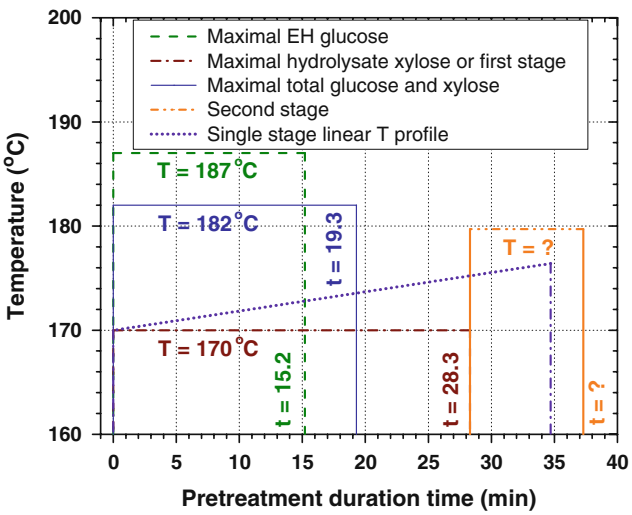

Fig. 4 An illustration of process optimization strategies for maximal recoveries of hemicellulose sugars and glucose from enzymatic hydrolysis using two-stage pretreatment

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