Effect of Organosolv Ethanol Pretreatment Variables on Physical Characteristics of Hybrid Poplar Substrates

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Abstract

Hybrid poplar (*Populus nigra* × *P. maximowiczii*) chips were pretreated using an organosolv ethanol process. The effect of pretreatment conditions (temperature, time, catalyst, and ethanol concentration) on the substrate characteristics, including fiber size, crystallinity, and degree of polymerization of cellulose, was investigated using an experimental matrix designed with response surface methodology. The conditions ranged 155–205°C, 26–94 min, 0.83–1.67% catalyst (H_2SO_4) on oven-dry wood chip (w/w), and 25–75% ethanol concentration (v/v). The results indicated that the substrate characteristics are controllable and predictable. Desirable substrates can be prepared by fine-tuning the processing parameters. The regression models developed, allowed the quantitative prediction of the substrate characteristics from the pretreatment conditions used.

Index Entries: Crystallinity; degree of polymerization; fiber size; hybrid poplar; organosolv ethanol pretreatment; substrate characteristics.

Introduction

Biorefinery of renewable resources for energy, chemicals, and materials has drawn considerable attention as the concerns for environmental impact and shortage of fossil petroleum are increasing. Fuel ethanol from lignocellulosics is one area receiving particular attention as the feedstocks are generally abundant, inexpensive, and renewable. A typical bioconversion process of lignocellulosics to ethanol consists of pretreatment, saccharification, fermentation, and ethanol distillation steps, whereas in the simultaneous saccharification and fermentation process, saccharification and fermentation are integrated into a single step. A primary techno-economic

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challenge in all lignocellulosics-to-ethanol bioconversion processes is the development of cost-effective pretreatment methods to make cellulose more accessible to enzymes *(1,2)*. The most adventitious strategy for an effective pretreatment is to develop a biorefinery platform where not only cellulose is recovered for bioethanol, but also lignin, hemicellulose, and extractive components of the lignocellulosic biomass are converted to valuable coproducts, which offsets the costs of feedstock pretreatment and enzymes for cellulose hydrolysis.

Among the various pretreatment methods, such as steam-explosion *(3)*, dilute acid pretreatment *(4,5)*, organosolv *(6,7)*, and others *(1)*, organosolv ethanol process was initially developed to make clean biofuel for turbine generators. Subsequent modification by the Canadian pulp and paper industry resulted in the Alcell® pulping process for hardwood *(8–10)*. Our initial results *(7)* indicated that the substrates pretreated by organosolv ethanol process from mixed softwoods possessed superior enzymatic digestibility over those pretreated by alternative processes, even at high residual lignin content. Furthermore, the process produces a particularly high-quality lignin fraction with potential in several industrial applications *(11)* and chemicals derived from hemicellulose *(12)*. Another advantage of the organosolv ethanol process is that the solvent (ethanol) used in the pretreatment is one of the final products of the bioconversion. Although the organosolv ethanol process has been largely investigated from the perspective of pulp and paper production from hardwood *(13–16)*, it is not well-studied as a pretreatment/biorefining tool for the bioconversion of lignocellulosic biomass. In particular, the effect of process parameters on the fractionation of major wood components, on the process mass balance, and on the chemical and physical characteristics of the substrate generated has not been investigated.

In previous work *(17)*, we investigated the pretreatment of hybrid poplar using organosolv ethanol process. The process was optimized from the perspective of recovery of cellulose, lignin, and hemicellulose. The enzymatic digestibility of the resulting substrate was also assessed. The organosolv ethanol lignin generated from the poplar was evaluated as an antioxidant *(18)*. In addition, the behaviors of the major wood components (lignin, hemicellulose, and cellulose) during the pretreatment, and chemical composition of resulting substrates and its dependence on process parameters were investigated as well *(17)*. The delignification was greatly dependent on ethanol concentration. Relatively lower ethanol concentration promoted acid-catalyzed cleavage of lignin, whereas higher ethanol concentration enhanced dissolution of depolymerized lignin fragments from the wood matrix. A maximum delignification was achieved at an ethanol concentration of about 70%((v/v). More hemicellulose was reserved in the substrate and less cellulose was depolymerized at high ethanol concentration, resulting in higher sugar recovery yield. However, retained hemicellulose and less hydrolyzed cellulose might have a negative

impact on enzymatic hydrolysability of the substrate. High-temperature and more catalyst promoted not only delignification but also degradation of cellulose and hemicellulose. Extra high-temperature could result in lignin condensation and further degradation of monosaccharides into furfural, hydroxymethylfurfural, and levulinic acid.

In the present research, physical characteristics influencing enzymatic hydrolysis of the organosolv substrates from hybrid poplar were investigated, including fiber size, crystallinity, and degree of polymerization (DP) of cellulose. The influence of the primary processing variables (temperature, time, catalyst, and ethanol concentration) on the substrate characteristics was discussed. Mathematical equations were regressed to predict quantitatively the effect of the processing variables on the substrate characteristics with the aid of response surface methodology and statistical analysis system (SAS) software (SAS Institute Inc., Cary, NC).

Materials and Methods

Organosolv Ethanol Pretreatment

Hybrid poplar (*Populus nigra × P. maximowiczii*) wood chips, screened to pass one-fourth of an inch (6.4 mm) round screen were generously provided by the National Renewable Energy Laboratory (NREL) (Golden, CO). The chemical composition of the poplar was reported previously *(17)*. Anhydrous ethanol used in pretreatment was purchased from Commercial Alcohol Inc. (Brampton, Ontario, Canada). As described schematically before *(17)*, the poplar chips were pretreated on a customer-designed four-vessel (2 L) digester manufactured by Aurora Products Ltd. (Savona, BC, Canada). Chips of 20.0 g (dry base) were pretreated, each batch in one vessel. After the pretreatment, substrate was separated from the processing liquor containing dissolved lignin and hemicellulose by filtration using nylon cloth. The substrate was first washed three times (300 mL × 3) with warm (60°C) aqueous ethanol of the same concentration as the processing liquor and then washed with warm water thoroughly. After being disintegrated in a standard British disintegrator (Robert Mitchell Co. Ltd., Montreal, Quebec, CA) for 5 min, the washed substrate was screened on a lab flat screen with 0.008 in. (0.203 mm) slits (Voith, Inc., Appleton, WI) to remove undefiberized wood chips (rejects). The screened substrate was stored at 4°C for characterization.

Characterization of Organosolv Ethanol Substrates

Viscosity measurement of the organosolv ethanol substrates from the poplar was conducted according to Technical Association of Pulp and Paper Industry Standard Method T230 om-99. The viscosity of a 0.5% cellulose solution in 0.5 *M* cupriethylenediamine was measured on a capillary viscometer (Cannon Instrument Co., State College, PA). The substrate

was carefully delignified before viscosity measurement, using sodium chlorite according to Useful Method G.10U of Pulp and Paper Technical Association of Canada.

Fiber length of the substrates was measured using a Fiber Quality Analyzer (LDA02, OpTest Equipment Inc., Canada). A dilute suspension of fibers with a fiber frequency of 25–40 EPS (events per second) was transported through a sheath low cell where the fibers are oriented and positioned. The images of fibers were detected by a built–in CCD camera, and the length of the fibers were measured by circular polarized light. The experiment was conducted according to the procedure described by Robertson et al. *(19)*. All samples were run in triplicate.

Crystallinity of the substrates was measured using a Bruker D8 Discover X-ray diffractometer (Madison, WI). X-ray radiation ($\lambda = 1.542$ Å) was generated by a Kristalloflex 780 generator with a Cu anode, powered at 40 kV and 20 mA. An air-dried fiber pad (approx 40 mm diameter and approx 1 mm thickness) was used for crystallinity measurement. The intensity was plotted against the scattering angle 2θ within a range of 5–40°. Two hundred scans were typically recorded for each sample. Crystallinity of the substrates was calculated according to the method described by Vonk *(20)* and Krassig *(21)*.

Experimental Design and Data Analysis

To investigate the influence of four process variables (temperature, reaction time at the temperature, acid catalyst dose, and ethanol concentration) on the characteristics of poplar substrates, an experimental matrix was designed using response surface methodology and a small Hartley composite design *(22)*. The matrix included 21 sets of conditions, including eight factorial points, eight star points, and five center points, as summarized in Table 1. The range of conditions examined was as follows: temperature, 155–205°C; time, 26–94 min; H_2SO_4, 0.83–1.67% of wood chips (w/w); and ethanol, 25–75% (v/v). The ratio of liquor to wood was constant (7 : 1, v/w) in all experiments. The condition set of the center point was temperature, 180°C; time, 60 min; H_2SO_4, 1.25% of wood chips (w/w); and ethanol, 50% (v/v). Data analysis (regression equations) was conducted and surface plots (figures) were prepared using SAS V9.0 for Windows (SAS Institute Inc.).

Results and Discussion

DP of Cellulose

The DP of cellulose is one of the substrate features that are known to influence the extent and rate of enzymatic hydrolysis. Some investigations concluded that DP is less important to enzymatic hydrolysis *(23,24)*, whereas other work indicated that substrates with low DP were hydrolyzed more

Table 1
Experimental Matrix of a Small Hartley Composite Design and Results

No.	Process condition[a]				Viscosity (mPaS)	Crystallinity (%)	Fiber length (mm)
	T (°C)	t (min)	S (%)	C (%)			
1	165	40	1.00	65	10.9	59	0.480
2	195	40	1.00	65	21.3	78	0.648
3	165	80	1.00	35	20.4	63	0.702
4	195	80	1.00	35	4.5	63	0.551
5	165	40	1.50	35	12.9	65	0.613
6	195	40	1.50	35	2.6	76	0.392
7	165	80	1.50	65	13.6	80	0.565
8	195	80	1.50	65	2.1	88	0.290
9	155	60	1.25	50	14.0	69	0.521
10	205	60	1.25	50	2.8	81	0.409
11	180	26	1.25	50	22.3	66	0.717
12	180	94	1.25	50	9.9	70	0.560
13	180	60	0.83	50	24.8	68	0.662
14	180	60	1.67	50	3.3	76	0.425
15	180	60	1.25	25	6.7	73	0.525
16	180	60	1.25	75	19.9	70	0.620
17	180	60	1.25	50	14.2	67	0.640
18	180	60	1.25	50	13.1	66	0.624
19	180	60	1.25	50	13.5	68	0.674
20	180	60	1.25	50	12.2	66	0.577
21	180	60	1.25	50	12.5	65	0.633

[a]T, temperature (°C); t, reaction time at the temperature (min); S, sulfuric acid dose (percent of dry wood chips [w/w]); C, concentration of aqueous ethanol (v/v [%]).

quickly and extensively than those with high DP *(25,26)*. It was believed that longer cellulose chains (high-DP) formed stronger networks by more extensive inter- and intramolecular hydrogen bonding, therefore limiting the accessibility of cellulose to the enzymes and thus diminishing the substrates susceptibility to hydrolysis *(25)*. In addition, the reduction of DP increased the number of cellulose chain ends available to the action of exoglucanase in the cellulase complex, thus generating high-reaction rate and glucose yield *(26–28)*.

The viscosity of the cellulose solution is an indirect measurement of cellulose DP. In the present research, the viscosity of the substrate solution in cupriethylenediamine was used as an indication of cellulose degradation (decrease in molecular weight). The data listed in Table 1 clearly indicate that the viscosity of the substrates greatly depended on process conditions, varying from 2.1 to 24.9 mPaS. The high-viscosity values are within the range of typical wood pulps for paper (15–25 mPaS) but much lower than the viscosity of cotton linters (>40 mPaS) *(29)*, whereas the low-values are too low to meet the strength requirement of pulp for paper. It is apparent that long reaction times reduced the viscosity because of the
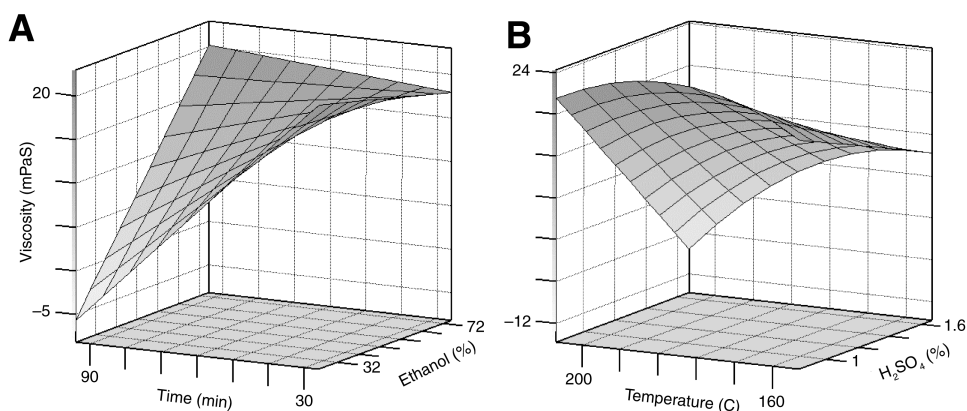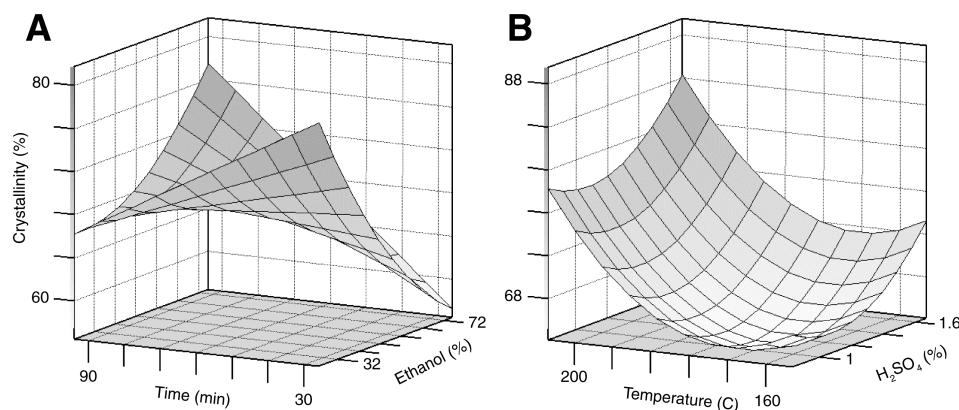
Fig. 1. Effect of process parameters on viscosity of the substrates prepared using organosolv ethanol process from hybrid poplar. The fixed variables were set at center point conditions. **A,** 180°C, 1.25% H_2SO_4; **B,** 60 min, 50%, ethanol.

acid-catalyzed depolymerization of cellulose when exposed to the acidic processing liquor at high-temperatures and, in particular, at low ethanol concentrations (Fig. 1A). Low ethanol concentration, which generates higher hydrogen ion concentration (lower pH value) at the same dose of H_2SO_4 *(30)*, promoted acid-catalyzed hydrolysis of cellulose. As expected, the substrate prepared at high ethanol concentrations had high-viscosity (Fig. 1A), implying that less depolymerization occurred. On the other hand, the substrates prepared at high-temperatures and sulfuric acid concentrations had low-viscosity (Fig. 1B), as the severe conditions enhanced the acidic hydrolysis of glucoside bonds in cellulose.

Crystallinity

The crystallinity of the cellulose is another substrate factor influencing enzymatic hydrolysis. However, contradictory conclusions have been drawn about the effect of crystallinity on enzymatic hydrolysis of cellulose *(24)*. Several studies found a positive correlation between crystallinity and hydrolysis and claimed that the amorphous part of cellulose was hydrolyzed first, leaving the more recalcitrant crystalline part unhydrolyzed *(23,31,32)*. These studies used relatively pure cellulosic substrates; the effect of other substrate features, such as residual hemicellulose and lignin contents, was minor or not considered. Other studies reported no correlation between crystallinity and the rate/extent of enzymatic hydrolysis of substrates. This was particularly true when real lignocellulosic substrates were used because other factors such as residual lignin and hemicellulose played more important roles in inhibiting hydrolysis *(25,33,34)*. In other words, the effect of crystallinity was overlapped in case of real substrate.

The effect of process parameters on the crystallinity of organosolv ethanol substrates is shown in Fig. 2 and Table 1. The crystallinity varied from 59% to 88% (Table 1), depending on processing parameters. Long
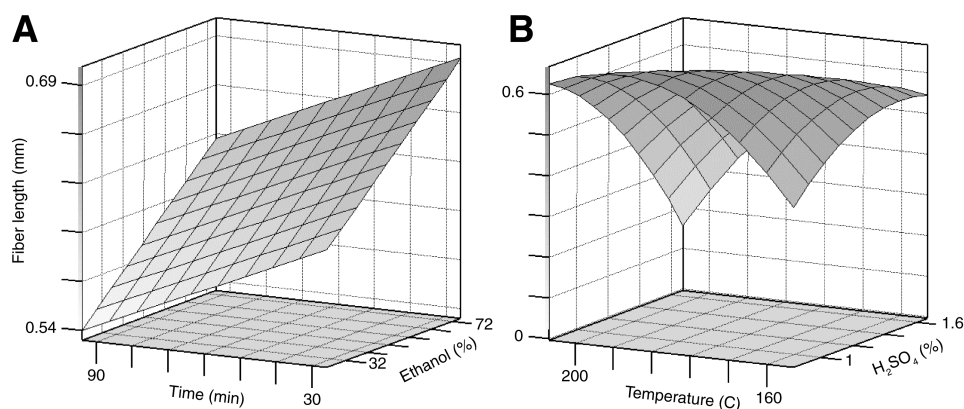
Fig. 2. Effect of process parameters on crystallinity of the substrates prepared using organosolv ethanol process from hybrid poplar. The fixed variables were set at center point conditions. **A,** 180°C, 1.25% H_2SO_4; **B,** 60 min, 50%, ethanol.

reaction time and relatively low ethanol concentration were expected to generate a substrate with high-crystallinity as these conditions favored the removal of amorphous lignin and hemicellulose. The results indicated that the substrates prepared at high ethanol concentration had lower crystallinity as more hemicellulose was retained in the substrates (right back corner of Fig. 2A). However, it is unclear why low-crystallinity was observed at extended time and low ethanol concentrations (left front corner of Fig. 2A). Figure 2B shows that the substrates generated at high-temperature and sulfuric acid concentration possessed higher crystallinity because of enhanced removal of amorphous lignin and hemicellulose. In addition, selective hydrolysis of amorphous part of cellulose might be another contributor to the high-crystallinity at high-temperature and catalyst dosage.

Fiber Size

It is believed that particle size is an important substrate factor influencing hydrolysis rate. In general, small particle size means large surface area available to enzymes, thus promoting hydrolysis. This is particularly true in case of "real" lignocellulosic substrates *(24)*. The effect of process variables on average length of substrate fibers is shown in Fig. 3 and Table 1. The length of shortest fibers (0.290 mm, no. 8) was only half of that of longer fibers (e.g., no. 17–21), as listed in Table 1. The fiber length of the poplar substrates was linearly related to ethanol concentration and reaction time, as shown in Fig. 3A. Long reaction times produced shorter fibers, whereas high-concentrations of ethanol generated longer fibers. High-temperatures and higher concentrations of sulfuric acid resulted in shorter fibers, as shown in Fig. 3B. The reduction of fiber length was primarily caused by "chemical cutting" occurring during the organosolv ethanol

Fig. 3. Effect of process parameters on average fiber length of the substrates prepared using organosolv ethanol process from hybrid poplar. The fixed variables were set at center point conditions. **A,** 180°C, 1.25% H_2SO_4; **B,** 60 min, 50% ethanol.
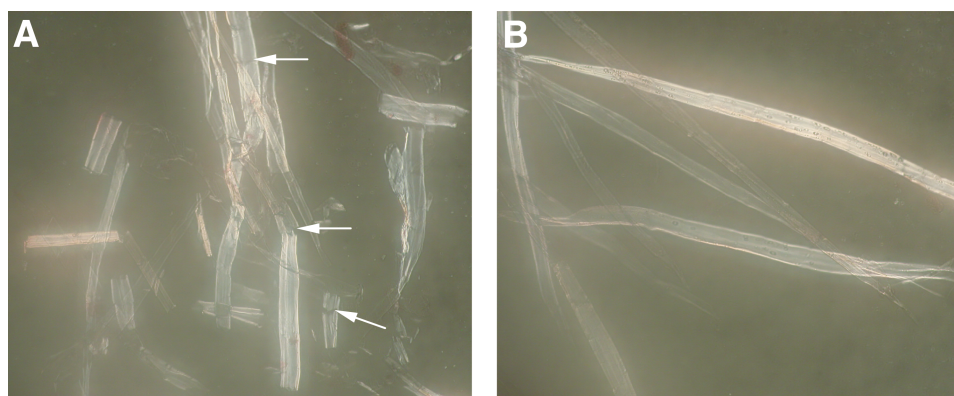
Fig. 4. Chemical cutting of fibers occurring during organosolv ethanol pretreatment observed under light microscope.

pretreatment, but the mechanism of the chemical cutting is not clear. The chemical cutting of the fibers was clearly observed under microscope.

Figure 4A shows the short pieces cut off from the fibers. Many cuttings are clearly visible along the fibers, as indicated by the arrows. The images of intact fibers are shown in Fig. 4B for comparison. It was observed that severe pretreatment conditions, for example, long reaction time, low ethanol concentration (high-acidity), high-temperature, and higher concentrations of acid catalyst, promoted the chemical cutting. Similarly, fiber cutting was observed in organosolv acetic acid pulping of wheat straw *(35)*. The chemical cutting is not desirable if the fibers are used for pulp and paper because the cutting weakens the strength of the fibers and papers.

However, from bioconversion point of view, the short fibers might be good substrates to enzymes because of the great surface area.

Regression Equations

The effect of pretreatment conditions on the physical characteristics of the poplar substrates was qualitatively discussed earlier. To quantitatively predict the effect of each process variable on the substrate features, the relationship between substrate features and process variables was fitted to a second-order polynomial equation using SAS software, as shown in Eq. 1.

$$Y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=1}^{k} a_{ij} X_i X_j \qquad (1)$$

where Y is the estimated value of the substrate features, k is the total number of independent variables (four in this case), X_is are the independent variables (temperature, time, catalyst dose, and ethanol concentration), the X_i, X_i^2, and $X_i X_j$ are terms describing linear, quadratic, and two-variable interaction effects, respectively, a_0 is a constant and a_i, a_{ii}, and a_{ij} are linear, quadratic, and interaction coefficients, respectively. The predictive models (Eqs. 2–4) developed for the substrate features are listed in Table 2. These equations can predict the substrate characteristics within the investigated range of process parameters. To validate the regression equations, the calculated values of the substrate characteristics using the equations at center point conditions were compared with the experimental data from five replicate pretreatment experiments. As listed in Table 2, the calculated values are in good agreement with the experimental data, implying the reliability of the equations.

Conclusion

The results of the present research indicated that the physical characteristics (DP of cellulose, crystallinity, and fiber length) of the organosolv ethanol substrates prepared from hybrid poplar are greatly dependent on the process variables (temperature, reaction time, ethanol concentration, and sulfuric acid dose). In other words, the substrates features are controllable and adjustable by fine-tuning the process variables to generate a desirable substrate for enzymatic hydrolysis. The regression equations developed provide a practical tool to quantitatively predict the substrate characteristics from the pretreatment variables. It is noteworthy that the optimization of the organosolv ethanol pretreatment must consider not only the hydrolysability of resulting substrate but also the recovery of cellulose, hemicellulose, and lignin. Severe processing conditions might generate a readily digestible substrate, but excessive

Table 2
Regression Equations for Prediction of Substrate Characteristics

Substrate feature	Equation[a]	Equation no.	R^2	Observed value	Calculated value
Viscosity (mPaS)	Viscosity (mPaS) = $-299.534 + 3.726T + 2.102t + 71.05S - 3.788C - 0.00911T^2 - 0.0115Tt - 0.543TS + 0.0139TC - 0.589tS + 0.0103tC + 0.736SC$	(2)	0.9787	13.1	13.0
Crystallinity (%)	Crystallinity (%) = $512.821 - 3.614T - 0.242t - 116.711S - 2.912C + 0.0111T^2 - 0.00917Tt + 0.00889TC + 1.126tS + 0.0113tC + 23.414S^2 + 0.00574C^2$	(3)	0.9764	66.4	66.2
Fiber length (mm)	Fiber length (mm) = $-12.249 + 0.108T + 0.0425t + 4.672S - 0.0246C - 0.000245T^2 - 0.000155Tt - 0.0171TS + 0.000147TC - 0.0125tS - 0.446S^2$	(4)	0.9215	0.63	0.58

[a]T, temperature (°C); t, reaction time (min) at the temperature; S, sulfuric acid dose (percent of dried wood chips [w/w]); C, ethanol concentration (v/v [%]).

depolymerization and decomposition of carbohydrate leading to low sugar recovery are undesirable.

Acknowledgment

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