Cost reduction and feedstock diversity for sulfuric acid-free ethanol cooking of lignocellulosic biomass as a pretreatment to enzymatic saccharification

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Abstract

We have previously demonstrated that a sulfuric acid-free ethanol (EtOH) cooking treatment enhances the enzymatic digestibility of eucalyptus wood and bagasse flour. In the present study, a reconfigured process that achieves similar performance was developed by identifying possible cost-competitive pretreatments that provide high cellulose-to-glucose conversion during subsequent enzymatic hydrolysis. The series of reconfigurations reduced EtOH usage in the pretreatment by more than 80% in comparison with our previous research. Higher initial pressures and intensive size reduction of the starting material are not required. The reconfigured process was applied to rice straw and Douglas fir, in order to confirm the feasibility of feedstock diversity.

Keywords: Sulfuric acid-free ethanol cooking; Bioethanol; Cost reduction; Feedstock diversity

1. Introduction

Dilute sulfuric acid-based chemical pretreatment (Cara et al., 2008; Knappert et al., 1980; Linde et al., 2008; Mosier et al., 2005; Sun and Cheng, 2002) is the most popular pretreatment method for lignocellulosic bioethanol production via enzymatic hydrolysis. This process, however, might have some undesirable effects: for example, the formation of aldehydes such as furfural via the degradation of the produced monosaccharides is essentially inevitable in this process; this in turn lowers the conversion yield of polysaccharides and inhibits the ethanol (EtOH) fermentation process. As an additional problem, sulfuric acid might corrode the reaction vessels. The recovery of the spent acid also complicates the downstream processing steps.

In recent years, a number of pretreatment methods other than the dilute sulfuric acid-based chemical pretreatment have been widely assessed (Chen and Liu, 2007; Kim et al., 2008; Silverstein et al., 2007). National Institute of Advanced Industrial Science and Technology (AIST) is developing an alternative sulfuric acid-free pretreatment that uses cost-effective pulverizing and cooking steps (Endo et al., 2006; Hideno et al., 2009; Lee et al., 2009; Teramoto et al., 2008a, 2008b). Recently, we examined a sulfuric acid-free EtOH cooking (SFEC) pretreatment (Teramoto et al., 2008a, 2008b), where cutter-milled lignocellulosic flours were exposed to an EtOH/water/acetic acid (AA) mixture in an autoclave. The heat required for the autoclave treatment can be essentially recovered by adequate process design. The SFEC system avoids the above mentioned problems originating from the use of a strong acid catalyst. Enzymatic hydrolysis experiments have demonstrated that the conversion of cellulosic components into glucose can reach ~100% under optimal conditions. The SFEC treatment does not

bring about intensive delignification, but improves the accessibility of enzyme to the cellulosic component in the original materials. Also, the cooking process involves less excessive degradation of pentosan. The in-feed EtOH is not substantially consumed by the formation of chemical bonding between EtOH and the wood components (Teramoto et al., 2008a). Therefore, it is considered that most of the EtOH feedstock can be recovered and reused in an adequately designed process. Diffractometory experiments have revealed that the conversion of the cellulosic crystal into an amorphous state is not a crucial factor for improving enzymatic digestibility (Teramoto et al., 2008a, 2008b). Field emission scanning electron microscopy has shown that SFEC induces the formation of pores ranging in size from approximately 10 to several 100 nm (Teramoto et al., 2008a, 2008b). It can be assumed that the porous surface is formed by the partial removal of lignin and hemicellulose, which improves the accessibility of the enzyme to the substrate.

While this earlier work was promising in terms of the superior enzymatic digestibility from the cellulosic fraction, the cost factors, including EtOH usage, processing pressure, and comminution, remain serious barriers to practical implementation. In the previous study, a tentative combination of optimal cooking conditions was as follows: EtOH/water in-feed weight ratio, 75/25; initial pressure, 5 MPa; temperature, 200°C; AA content, 1 wt% relative to the cooking liquor (Teramoto et al., 2008a). The feasibility of feedstock diversity also posed a technical challenge. The present study was therefore initiated to identify possible cost-competitive pretreatments that can provide high cellulose-to-glucose conversion during the subsequent enzymatic hydrolysis not only for eucalyptus but also for softwood (Douglas fir) and herbal plants (bagasse and rice straw).

2. Materials and Methods

2.1 Samples and Solvents

Eucalyptus and Douglas fir wood chips (major axis, 25–50 mm; minor axis, 10–20 mm; thickness, 2–5 mm) were purchased from Oji Paper Co. Ltd., Tokyo, Japan. Bagasse and rice straw were provided by Japan Planning Organization Inc., Tokyo, Japan, and the specified nonprofit corporation Shimane Bioethanol Workshop, Shimane, Japan, respectively. The original materials were cutter-milled to pass through 0.2, 2, or 5-mm pore-size sieves. The flour samples were stored at 20°C and 60% relative humidity before use. EtOH, AA, and other chemicals used in this study were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan; these were all guaranteed to be of reagent grade and were used without further purification.

2.2 Holocellulose, α-Cellulose, and Lignin Determinations

The biomass flour (particle size, <0.2 mm) was dried at 40°C for 24 h in vacuo, extracted in a Soxhlet extractor with an EtOH/toluene solution (1/2 by volume) for 6 h, and dried at 40°C for 24 h in vacuo.

The holocellulose content was determined as the amount of $NaClO_2$-delignified residue (Wise et al., 1946): 2.5 g of defatted biomass flour was heated with 150 mL water containing 0.2 mL acetic acid and 2.0 g $NaClO_2$ for 1 hour at 75°C. 0.2 mL acetic acid and 1.0 g $NaClO_2$ were added into the system each hour until a total of 0.8

mL acetic acid and 5.0 g $NaClO_2$ have been added. The delignified product, holocellulose, was filtered, washed with distilled water and acetone, dried at 105°C for 24 h in vacuo, and weighed.

The α-cellulose content was determined as the amount of residue insoluble in a 17.5% NaOH aqueous solution according to TAPPI 203: 25 mL of a 17.5% NaOH aqueous solution was added to a flask containing 1 g of the obtained holocellulose mentioned above. The mixture was stirred at 20°C for 40 min, and 25 mL of distilled water was added to it. After 5 min, the residue was filtered, and 40 mL of a 10% AA aqueous solution was then added to the residue. The residue was filtered again and washed with 1 L of boiling water. The α-cellulose residue was filtered, dried at 105°C for 48 h in vacuo, and weighed.

The Klason lignin content was determined as the amount of residue insoluble in a 72% sulfuric acid aqueous solution (Browning, 1967) as follows: to 1 g of defatted biomass flour, 15 mL of a 72% sulfuric acid aqueous solution was added. The mixture was stirred at 20°C for 4 h. Subsequently, 560 mL of distilled water was added to it, and the system was refluxed for 4 h. Thereafter, the residue was filtered, washed with boiling water and cold water, dried at 105°C for 24 h in vacuo, and weighed.

The dry-basis contents of holocellulose, α-cellulose, and Klason lignin in the original materials were calculated using their respective weights. The α-cellulose content was considered the cellulose content. The hemicellulose content was determined by subtracting the α-cellulose content from the holocellulose content.

The compositions of the feedstocks used in this study are presented in Table 1.

2.3 Cooking

A sample of the humidity-conditioned biomass flour (net flour content, 2–10 g) in aqueous EtOH (EtOH/water in-feed weight ratio, 90/10, 75/25, 50/50, 25/75, and 10/90) was heated at 120°C–220°C for 0–60 min in the presence of 0%–20% AA. The liquor-to-wood weight ratio was varied between 1:1 and 5:1. The cooking experiments were performed in a 57-mL stainless steel (SUS316) autoclave (Nitto Koatsu Co. Ltd., Tsukuba, Japan) equipped with a motor-driven electromagnetic stirrer (300 rpm). The biomass flour along with the designated amounts of reagents was introduced into the autoclave. After purging with nitrogen gas and regulating the pressure (0.1–5 MPa), the reactor was plunged into a mantle heater at 25°C. The reactor was heated at a rate of 10°C/min up to a prescribed temperature, and the reaction pressure was gradually increased in parallel to 1.5–8 MPa. After the temperature of the system reached the specified temperature, cooking was conducted over a selected time period, defined as the reaction time of cooking. In this paper, the term "reaction time" excludes the warm-up period. Subsequently, the reactor was immediately immersed in a water bath at room temperature. The resultant products were separated from the cooking liquor by filtration and washed with 100 mL of EtOH/water (composition identical to that of the respective cooking liquor) or 10, 20, 50, and 100 mL of water. The cooked products were stored overnight at 25°C in the atmosphere. The cooking liquor and washes were combined and retained for further analysis.

2.4 Material Balance Determinations

The 5-hydroxymethyl furfural (HMF) and furfural contents in the cooking liquors

were determined using a CCP & 8020 HPLC system (Tosoh Corporation, Tokyo, Japan) equipped with an Aminex 87H column (Bio-Rad Laboratories K. K., Tokyo, Japan) at 40°C; 8 mM H_2SO_4 was used as the eluent at a flow rate of 0.6 mL/min. Aliquots (20 μL) appropriately diluted with distilled water were injected into the HPLC apparatus. The remainder of the diluted cooking liquors was condensed, and the residue was dissolved in water. We conjectured that the water-soluble fraction was derived from the partially decomposed hemicellulose in the original biomass flour. On the other hand, the water-insoluble fraction was assumed to be lignin eluted in the cooking liquor. After the respective parts were dried at 40°C in vacuo, they were weighed.

2.5 Enzymatic Hydrolysis

The enzyme used for hydrolysis was a commercial product "Meicelase," derived from the fungus *Trichoderma viride*, which was supplied by Meiji Seika Co. Ltd., Tokyo, Japan. Enzymatic hydrolysis experiments were routinely performed with ca. 60 mg (net weight, 50 mg) of pretreated wood in 17 mL of 50 mM acetate buffer (pH 5.0) containing 2 mg of enzyme. The enzyme loading corresponds to 9.5 FPU/g-pretreated solid. The mixtures were incubated at 45°C in a rotary shaker set at 250 rpm and sampled periodically. The samples were stored at −18°C and then heated at 95°C for 15 min and centrifuged; the supernatants were retained for subsequent sugar analysis. The amount of glucose in the supernatant was estimated using an LC-2000Plus HPLC system (JASCO Corporation, Tokyo, Japan) equipped with an Aminex HPX-87P column (Bio-Rad Laboratories K. K., Tokyo, Japan) at 80°C; distilled water was used as the eluent at a flow rate of 1 mL/min. In the present study,

100 % conversion (degree of cellulose saccharification) represents complete digestion of α-cellulose in the original materials.

2.6 Severity Parameters

A severity parameter can be used to unify the data obtained for different combinations of temperature, time, and catalyst concentration. The severity parameter for water-only hydrolysis is defined as follows:

$$R_0 = t \exp\left[\frac{T-100}{14.75}\right],$$

where t is the reaction time (min), and T is the hydrolysis temperature (°C) (Overend and Chornet, 1987). We can derive a combined severity parameter that includes time and temperature parameters and the hydrogen ion activity parameters as (Chum et al., 1990)

$$R_0' = [\mathrm{H}^+] t \exp\left[\frac{(T-100)}{14.75}\right],$$

where $[\mathrm{H}^+]$ was determined from the pH of the cooking liquor at 20°C for the solutions under the pretreatment conditions.

3. Results and Discussion

3.1 Reconfiguration of Cooking Conditions

In previous research, highly enzymatically digestible eucalyptus and bagasse

substrates were produced by SFEC pretreatment using the following tentative optimal cooking conditions: EtOH/water in-feed weight ratio, 75/25; initial pressure, 5 MPa; temperature, 200°C; AA content, 1 wt% relative to the cooking liquor. However, the high EtOH usage and pressure are serious barriers to the practical application of that process. In the present study, we first developed a reconfigured process that can achieve similar performance levels but reduces the cost of the cooking process.

SFEC treatments for cutter-milled eucalyptus flour (particle size, <0.2 mm) were conducted with the cooking liquors having different EtOH/water ratios (90/10, 75/25, 50/50, 25/75, and 10/90) under the tentative optimal conditions (except the EtOH/water ratio); the results are listed in Table 2. Here, we conjectured that the water-soluble and water-insoluble fractions were derived from partially decomposed hemicellulose and lignin, respectively. As shown in Table 2, lignin removal decreased with an increase in the water content in the cooking liquor, while hemicellulose removal was not remarkably affected by the EtOH/water ratio. HMF and furfural formation increased with water dosage. However, high conversion of cellulose to glucose (≥95 %) was observed in the enzymatic hydrolysis experiments with EtOH/water ratios of 75/25, 50/50, and 25/75. Under further water-rich systems (EtOH/water = 10/90 and 0/100), however, the digestibility of the substrates was low. Under these two sets of conditions, excessive degradation of the samples occurred, as confirmed by the higher HMF and furfural formation. This can be attributed to the decrease in pH with increasing water content. Since the formation of HMF originated from C6-sugars being decomposed, there is a loss of glucose during the pretreatment. However, the HMF formation was small and less than 1.5 wt% of the original materials for the pretreatment with EtOH/water ratios of 90/10, 75/25, 50/50, and 25/75. For highly

enzymatically digestible products pretreated, the sum of glucose and HMF formations almost corresponded to the α-cellulose content in the raw material.

Among the EtOH/water ratios employed here, we considered 25/75 to be the most appropriate for reduced EtOH usage as well as higher digestibility. Compared to our previous study (Teramoto, 2008b) using combined pretreatment of SFEC for eucalyptus wood chips and subsequent ball-milling, some of the results seem paradoxical. Namely, the comparison reveals that in the previous study under similar conditions an EtOH/water ratio of 75/25 was the optimum whereas the degrees of enzymatic saccharification of cellulose were 87 and 27 % for 90/10 and 25/75 (Teramoto et al., 2008b). The discrepancy can be explained by the difference of the progression manners of pretreatment between chip and flour. EtOH in the cooking liquor mainly elutes the partially degraded lignin component and removes that. On the other hand, by using water-rich cooking liquor, parts of hemicellulose and lignin are disrupted during the pretreatment into forms that precipitate onto the solids. In the case of the pretreatment of wood flour, the precipitates interfere less with enzyme action than native forms of hemicellulose and lignin as described later. For wood chips, however, since the precipitates reduced the cooking efficiency for the inside of the chips, further cooking effect could hardly be accomplished. Therefore, in our previous result (Teramoto 2008b) treating eucalyptus chips, EtOH-rich cooking liquor was more effective due to its elutability of the wood component. On the other hand, as shown in our previous report (Teramoto 2008b), ball-milling itself has useful role in the improvement of enzymatic digestibility of biomass. Under the EtOH-rich condition (EtOH:water in-feed ratio, 90/10), the small amount of water was ineffective for the improvement of the enzymatic digestibility and the subsequent ball-milling is needed.

Therefore, it is not adequate to simply compare the cooking condition of the combined process of SFEC and ball-milling with that for the present SFEC of wood flour.

Another reconfiguration of the cooking conditions was made to address the initial pressure, which is a major plant cost factor. A wide-ranging examination was conducted for eucalyptus flour (particle size, <0.2 mm) under different initial pressures (0.1, 0.5, and 5 MPa). Extensive data were collected with different EtOH/water ratios (75/25, 50/50, and 25/75), temperatures (120–200°C), durations (0–60 min), and AA contents (1–5%). The resultant degree of enzymatic saccharification of the cellulose in the pretreated products is represented as a function of $\log R_0'$ in Figure 1. For the calculation of $\log R_0'$, pH of the cooking liquor at 20°C was determined before the pretreatment. The pH values after cooking were 0.2–0.3 smaller than those before the pretreatment due to formation of acetic acid from degradation of hemicellulose component. As shown in this figure, the degree of saccharification correlated well with $\log R_0'$, reaching ≥90 %-saccharification at $\log R_0' = 1–1.5$ irrespective of the initial pressure. Therefore, a higher initial pressure is not absolutely necessary for improved enzymatic digestibility of eucalyptus flour.

As listed in Table 2, $\log R_0'$ can be increased by an increase in the water dosage in the cooking liquor even if other energy-consuming factors (time and temperature) are identical. Therefore, the ratio EtOH/water = 25/75 is the most appropriate combination in this regard as well. During the treatment, water acts as a decomposing agent for a part of the hemicellulose and lignin at high temperatures and pressures. However, EtOH is mainly responsible for the dissolution of the hydrophobic component, lignin. The EtOH/water in-feed weight ratio of 25/75 is the most balanced combination and can be regarded as the most effective activator for enzymatic

hydrolysis.

Even though some of the hemicellulose and lignin were eluted by the cooking process, the degrees of removal do not lead directly to high enzymatic digestibility. In Figure 2, the extensive saccharification data are represented as functions of the removals of hemicellulose and lignin. In contrast to the high degree of correlation in Figure 1, the degree of saccharification is less correlated with the hemicellulose removal and hardly related with lignin removal. As has been suggested by our previous studies (Teramoto et al., 2008a, 2008b), the removals of hemicellulose and lignin estimated as soluble parts in the cooking liquor are not the most important factors for the improvement of the enzymatic digestibility of lignocellulosics. Hemicellulose and lignin are disrupted during the pretreatment into forms that precipitate onto the solids but interfere less with enzyme action than native forms of hemicellulose and lignin. Such a concept is consistent with microscopic observations of pretreated biomass surfaces, where the treatment induced the formation of pores with a size of a few tens nanometers (Teramoto et al., 2008a, 2008b). The porous surface improved the accessibility of the enzyme to the substrate.

Subsequently, we attempted to further reduce the EtOH usage. The initial EtOH dosage can be decreased not only by regulating the EtOH/water ratio but also by reducing the cooking liquor/feedstock ratio. Table 3 lists the properties of the products pretreated with different liquor/feedstock weight ratios (1, 2, 3, and 4). When the ratio was 1 and 2, relatively lower degree of cellulose saccharification was observed. However, when the liquor/feedstock ratio was 3, digestibility reached as high as ~100%. Therefore, this series of reconfigurations attained a reduction of 80% in EtOH usage by just modifying the cooking conditions in comparison with the system using the previous

tentative optimal conditions (EtOH/water in-feed weight ratio, 75/25; cooking liquor/feedstock weight ratio, 5) (Teramoto et al., 2008a).

EtOH usage can also be further reduced by reconfiguring the washing process conducted after cooking. In the previous study, the cooking products were filtered and the solid parts were washed with EtOH and/or aqueous EtOH (Teramoto et al., 2008a, 2008b). In this study, the filtered solid products were rinsed with different amounts of water instead of EtOH or aqueous EtOH. Here, the cooking treatment was performed under the following optimal conditions: EtOH/water in-feed weight ratio, 25/75; temperature, 200°C; AA content, 1 wt% relative to the cooking liquor. Although the removals of hemicellulose and lignin decreased with the volume of rinse water as listed in Table 4, the degree of saccharification (48 h) was found to be 95.2% for the least amount of rinse water (10 mL for 2-g feedstock loading). Therefore, the usage of EtOH for washing after cooking was found to be unnecessary. Moreover, just a small amount of water was found sufficient for the washing process.

3.2 Particle Size

Size reduction is a necessary preliminary step in order to obtain adequate yields in the production of EtOH from lignocellulosics by any of the available methods. Usually, the particle size of feed materials is 10–50 mm after chipping and 0.2–10 mm after milling or grinding. The power requirements of mechanical comminution depend on the final particle size and the biomass characteristics (Cadoche and Lopez, 1989). The maximum energy input in the size reduction step should be lower than 30 kWh per tonne of processed raw material, provided the maximum final particle size is kept in the

range of 3–6 mm (Cadoche and Lopez, 1989). Figure 3 illustrates the enzymatic hydrolysis behaviors using other types of eucalyptus flour (particle sizes, <2 or <5 mm), wherein the SFEC treatment was conducted under the optimum conditions for eucalyptus flour particles of less than 0.2 mm in size, thereby demonstrating the effect of the particle size of the original material. Although the removal of hemicellulose and lignin slightly decreased in comparison with that with the <0.2-mm particles as listed in Table 5, ≥95%-saccharification of cellulose occurred for each sample after 48-h enzymatic treatment. In the initial stage (≤24 h) of the enzymatic hydrolysis, higher degrees of cellulose saccharification were observed the products cooked under severer conditions (smaller particle size and higher temperature). However, taking into consideration the eventual high yield of glucose even when using <5-mm particles, this cooking pretreatment is a realistic approach. As listed in Table 6, the cooked feedstock reached a degree of saccharification of 95.5% of the cellulose (48 h) even with the mildest combination of the cooking conditions employed in the present study (EtOH/water ratio, liquor/feedstock ratio, washing, and particle size).

3.3 Feedstock Diversity

In order to examine feedstock diversity, we conducted pretreatment of rice straw, bagasse, and Douglas fir. Both the herbal feedstocks (rice straw and bagasse) could be easily converted into highly digestible materials by pretreatment with the mildest conditions estimated for eucalyptus. The conditions are as follows: particle size, <5 mm; cooking liquor/feedstock weight ratio, 3; EtOH/water ratio, 25/75; temperature, 200°C; time, 60 min; AA content, 1 wt%; initial pressure, 0.1 MPa. In contrast,

severer conditions (AA content and initial pressure) were required to improve the enzymatic digestibility of softwood (Douglas fir). Figure 4 presents plots of the enzymatic digestibility of pretreated Douglas fir flour (<2 mm) as a function of logR_0'. In comparison with Figure 1, higher logR_0' is required to increase the enzymatic digestibility of Douglas fir. Here, the conditions for higher logR_0' correspond to a larger AA loading (~20 wt%), where the pH in the cooking system is decreased. The initial pressure also affects the digestibility; it was possible to decrease this from 5 to 2 MPa for higher logR_0'. Therefore, for Douglas fir, it is necessary to increase the initial pressure as well as acidity of the cooking system, suggesting the presence of a shielding structure in this softwood feedstock mainly due to the condensed type nature of softwood lignin. As shown in Figure 5, the removals of hemicellulose and lignin estimated as soluble parts in the cooking liquor are not strongly related with the enzymatic digestibility of Douglas fir, in analogy with the case of eucalyptus.

4. Conclusions

The SFEC pretreatment conditions were reconfigured, mainly for eucalyptus, in order to identify cost-effective SFEC pretreatment conditions that can provide high cellulose-to-glucose conversion during the subsequent enzymatic hydrolysis. The reconfiguration of parameters, including EtOH usage, processing pressure, and comminution, allowed a reduction in both the processing and capital costs without sacrificing enzymatic digestibility. The reconfigured process could be applied to herbal plants (bagasse and rice straw). However, slightly severer conditions (AA

content and initial pressure) were required to improve the enzymatic digestibility of softwood (Douglas fir).

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Figure captions

Figure 1. Effect of a severity parameter on enzymatic digestibility (48 h) of the cellulose fraction of SFEC-treated eucalyptus flour (particle size, <0.2 mm).

Figure 2. Effect of (a) hemicellulose and (b) lignin removals of SFEC-treated eucalyptus flour (particle size, <0.2 mm) on enzymatic digestibility (48 h) of cellulose fraction. Removal of hemicellulose and lignin is represented in terms of wt% of the respective content in the original flour.

Figure 3. Enzymatic hydrolysis behavior of SFEC-treated eucalyptus flour (particle size, <2 mm or <5 mm), showing the effects of particle size and cooking temperature. SFEC conditions: EtOH/water, 25/75 by weight; liquor/feedstock, 5 by weight; cooking temperature, 180 or 200°C; cooking time, 60 min; AA content, 1 wt%-cooking liquor; initial pressure, 0.5 MPa.

Figure 4. Effect of a severity parameter on enzymatic digestibility (48 h) of cellulose fraction of SFEC-treated Douglas fir flour (particle size, <2 mm).

Figure 5. Effect of (a) hemicellulose and (b) lignin removals of SFEC-treated Douglas fir flour (particle size, <2 mm) on enzymatic digestibility (48 h) of cellulose fraction. Removal of hemicellulose and lignin is represented in terms of wt% of the respective content in the original flour.

Table 1 Percentage composition on dry-weight basis for the feedstocks used in this study

Component	Eucalyptus	Douglas fir	Bagasse	Rice straw
Holocellulose	76.3	75.7	63.2	56.6
α-Cellulose	42.2	47.3	30.7	26.5
Hemicellulose	34.1	28.4	32.5	30.1
Klason lignin	28.1	25.3	22.8	26.3
Extractives	1.4	1.7	6.4	4.1
Ash	0.6	0.3	2.3	14.9

Table 2 Results of SFEC treatment for eucalyptus flour, describing the effect of EtOH/water ratio (initial particle size, <0.2 mm; liquor/feedstock, 5 by weight; cooking temperature, 200°C; cooking time, 60 min; AA content, 1 wt%-cooking liquor; initial pressure, 5 MPa)

EtOH/water (wt/wt)	logR_0'	Water-soluble fraction (wt%-original flour)	Water-insoluble fraction (wt%-original flour)	HMF formation (wt%-original flour)	Furfural formation (wt%-original flour)	Degree of cellulose saccharification (48 h) (mol%)
90/10	0.753	8.6	20	0.20	0.37	16.9
75/25	0.929	12	20	0.50	0.56	96.6
50/50	1.50	10	23	0.85	1.1	98.4
25/75	1.92	11	4.2	1.3	1.8	~100
10/90	2.14	12	0.44	2.4	1.9	69.7
0/100	2.34	11	0.03	3.6	2.1	46.4

Table 3 Results of SFEC treatment for eucalyptus flour, describing the effect of liquor/feedstock in-feed ratio (initial particle size, <0.2 mm; EtOH/water, 25/75 by weight; cooking temperature, 200°C; cooking time, 60 min; AA content, 1 wt%-cooking liquor; initial pressure, 0.5 MPa)

Liquor/feedstock (wt/wt)	Water-soluble fraction (wt%-original flour)	Water-insoluble fraction (wt%-original flour)	Degree of cellulose saccharification (48 h) (mol%)
4	8.7	3.2	~100
3	7.4	2.3	~100
2	3.1	0.20	88.1
1	2.0	0.17	51.4

Table 4 Results of SFEC treatment for eucalyptus flour, describing the effect of the amount of rinse water (initial particle size, <0.2 mm; liquor/feedstock, 5 by weight; EtOH/water, 25/75 by weight; cooking temperature, 200°C; cooking time, 60 min; AA content, 1 wt%-cooking liquor; initial pressure, 0.5 MPa)

Rinse water (mL)	Water-soluble fraction (wt%-original flour)	Water-insoluble fraction (wt%-original flour)	Degree of cellulose saccharification (48 h) (mol%)
100	9.2	0.17	~100
50	9.1	0.12	~100
20	9.0	0.05	~100
10	7.8	0.02	95.2

Table 5 Results of SFEC treatment for eucalyptus flour, describing the effect of particle size of feedstock (EtOH/water, 25/75 by weight; liquor/feedstock, 5 by weight; cooking temperature, 180 or 200°C; cooking time, 60 min; AA content, 1 wt%-cooking liquor; initial pressure, 0.5 MPa)

Particle size (mm)	Cooking temp. (°C)	Water-soluble fraction (wt%-original flour)	Water-insoluble fraction (wt%-original flour)
<2	200	11.9	2.8
<5	200	10.8	2.2
<2	180	10.5	3.8
<5	180	8.6	3.3

Table 6 Exploring optimal SFEC pretreatment conditions for eucalyptus flour (EtOH/water, 25/75 by weight; cooking time, 60 min; AA content, 1 wt%-cooking liquor; initial pressure, 0.1 MPa; rinse water, 10 mL)

Particle size (mm)	Liquor/feed stock (wt/wt)	Cooking temp. (°C)	Water-soluble fraction (wt%-original flour)	Water-insoluble fraction (wt%-original flour)	Degree of cellulose saccharification (48 h) (mol%)
<2	4	200	6.89	0.25	~100
<5	4	180	5.75	0.20	96.7
<2	3	200	5.04	0.03	97.5
<5	3	180	4.62	0.02	95.5

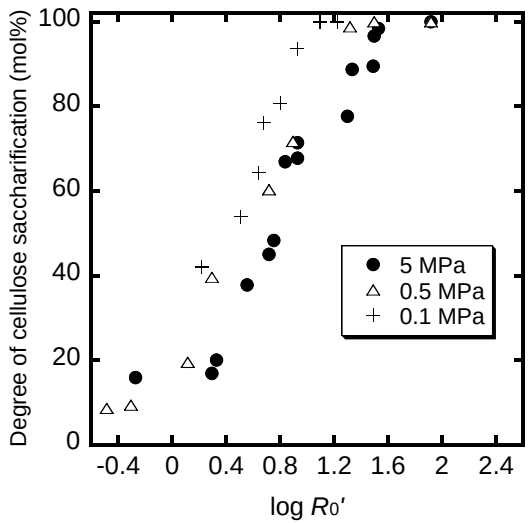
Degree of cellulose saccharification (mol%)
100
80
60
40
20
0
-0.4
0
0.4
0.8
1.2
1.6
2
2.4
log R_0'
5 MPa
0.5 MPa
0.1 MPa

Figure 1

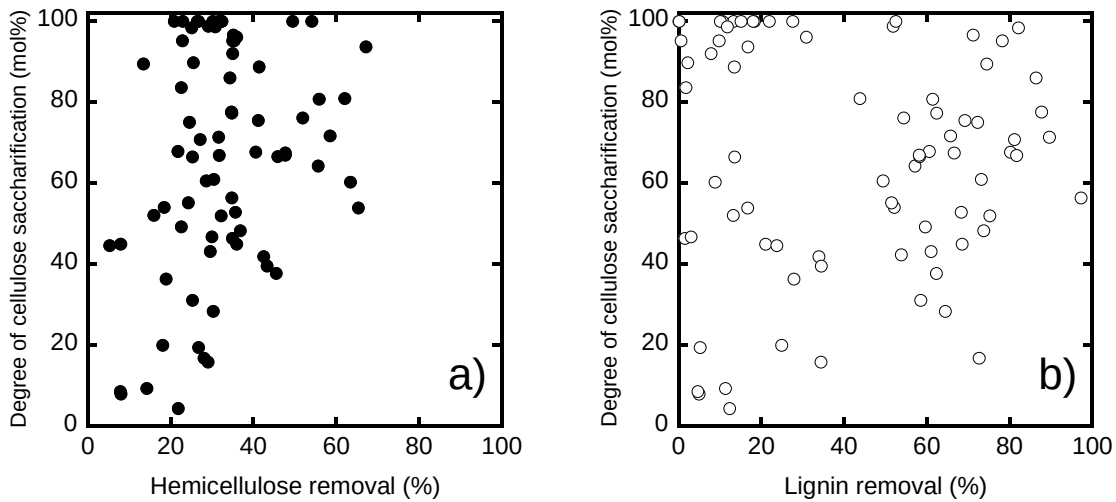
Degree of cellulose saccharification (mol%)
Hemicellulose removal (%)
a)
Lignin removal (%)
b)
0
20
40
60
80
100

Figure 2

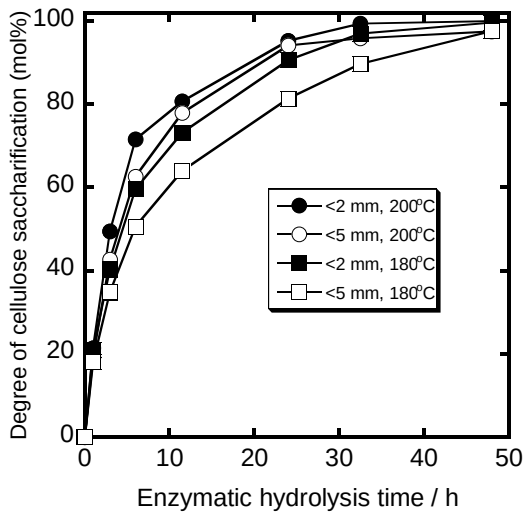
Degree of cellulose saccharification (mol%)
100
80
60
40
20
0
0
10
20
30
40
50
Enzymatic hydrolysis time / h
<2 mm, 200°C
<5 mm, 200°C
<2 mm, 180°C
<5 mm, 180°C

Figure 3

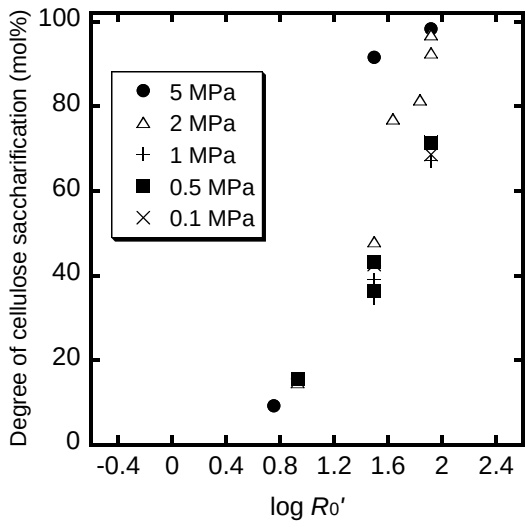
Degree of cellulose saccharification (mol%)
100
80
60
40
20
0
-0.4
0
0.4
0.8
1.2
1.6
2
2.4
log R_0'
5 MPa
2 MPa
1 MPa
0.5 MPa
0.1 MPa

Figure 4

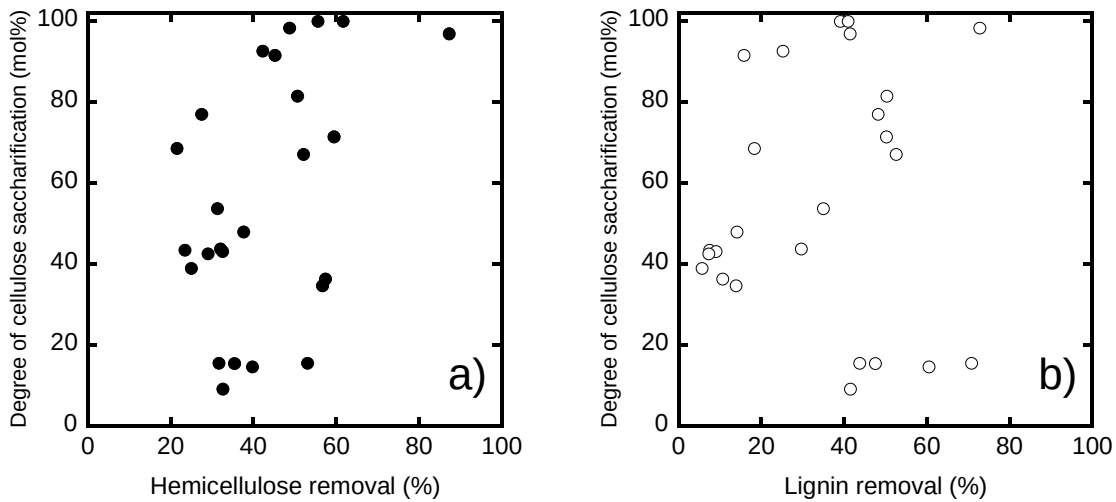
Degree of cellulose saccharification (mol%)
Hemicellulose removal (%)
a)
Lignin removal (%)
b)
0
20
40
60
80
100

Figure 5