Effects of residual lignin and cellulose swelling on the rate of enzymatic saccharification of softwood and hardwood acid sulfite pulps

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Abstract
We estimated the effect of residual lignin and pulp swelling on the rate of enzymatic saccharification to increase production of ethanol from acid sulfite pulp (SP) by means of enzymatic treatment. The resolution ratio of hardwood (Acacia mearnsii) SP after the enzymatic treatment was lower compared to softwood (Larix leptolepis) SP even though lignin content of hardwood SP was lower. The pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) analysis revealed that the residual lignin in hardwood SP could more easily adsorb enzyme compared to softwood SP, and the residual lignin in hardwood SP should interfere with the binding of the enzyme to cellulose. The beating treatment of pulp increased the swelling of pulp. The enzymatic saccharification rate was increased by the beating treatment. On the other hand, the delignification treatment was more effective than the beating treatment at enhancing enzymatic saccharification of both hardwood and softwood SPs. We found that the delignification process should be considered a high-priority technique for enhancing enzymatic saccharification of SP.

Keywords: Acid sulfite pulp, Enzymatic saccharification, Cellulase, Amount of enzyme adsorbed onto lignin

Introduction
The wood refinery process, which creates fuels and chemicals by utilizing wood, has attracted increasing attention. Some currently operational acid sulfite pulp (SP) mills can produce various chemicals and materials from SP and spent sulfite liquor (SSL). For example, wood chips (oven-dried 1000 kg) as raw materials provide 500 kg of cellulose as pulp and 300 kg of lignosulfonate, 3 kg of vanillin, and 50 kg of ethanol, which are obtained by utilizing carbohydrates and lignin in SSL [1, 2]. We also have reported the ways to enhance ethanol production by Pichia stipitis from pentose and oligosaccharides in SSL by removing acetic acid from SSL using a combined treatment with calcium oxide and an ion exchange resin [3, 4]. Therefore, acid sulfite cooking, which is an established process in the pulp and paper industry, is one of the most notable technologies for the wood refinery process.

Ethanol is one of the most important types of fuel, and can be produced from wood via a refinery process. The pretreatment process for removal of lignin from wood is necessary for enzymatic saccharification of cellulose. Several methods have been proposed for optimization of the pretreatment in enzymatic saccharification. The acid sulfite cooking is a feasible technique for production of glucose from pulp using enzymatic saccharification. The SP can be hydrolyzed to glucose using enzymatic treatment. The resulting glucose from pulp can serve as a raw material for ethanol production. We found that, for Japanese larch (Larix leptolepis) SP, the resolution ratio of pulp subjected to enzymatic treatment is higher compared to kraft pulp and soda–anthraquinone (AQ) pulp,
and that this resolution ratio is independent of kappa number of SP in the range of 20–60 [5]. The pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) method has been successfully used for estimating the amount of both enzyme and lignin in enzyme-treated pulp [6]. Takahashi and Ohi [6] have determined two ion peaks with $m/z = 117$ and $130$ obtained from enzyme-treated pulp by Py-GC/MS were indole and methyldol, respectively, which are typical degradation products of tryptophan. They also have reported that there is a positive correlation between nitrogen content of the enzyme-treated pulp by elemental analysis and the amount of enzyme determined by Py-GC/MS [6]. H'ng YY et al. [7] isolated the residual lignin in alkali pulps prepared from oil palm (Elaeis guineensis) empty fruit bunch (EFB) using an enzymatic method, and characterized it by Py-GC/MS. The lignin from the enzyme-treated EFB pulp was further purified by washing and they reported that the amount of enzyme adsorbed onto the lignin in the pulp analyzed by Py-GC/MS was decreased by the purification [7]. These results indicated the impurity compounds were removed from the enzyme-treated pulp, and it has been strongly suggested that the ion peaks with $m/z = 117$ and $130$ were derived from the enzyme, not from other compounds. We assumed that the difference of resolution ratio of enzyme-treated SP and soda–AQ pulp is due to the difference between pulps in the amount of enzyme adsorbed onto the residual lignin. It was still unclear, however, whether residual carbohydrates of the pulp still adsorb the enzyme even if the pulp is thoroughly washed with water. Linder and Teeri reported that the interaction of the cellulose-binding domain (CBD) with cellulose was fully reversible and that CBD could be eluted from cellulose by means of simple dilution with a buffer solution [8]. We have also applied to wash the enzyme-treated pulp by soaking in acetate buffer to release the enzyme from cellulose in the enzyme-treated pulp, in order to evaluate the amount of enzyme adsorbed onto lignin using the Py-GC/MS method [9]. We have reported that the amount of enzyme adsorbed onto residual lignin in larch SP was lower than that in larch soda–AQ pulp [9].

The structure of lignin in hardwood differs from that in softwood. The structure of the residual lignin in pulp should affect the adsorption of enzyme to lignin and the enzymatic saccharification efficiency of the pulp. To achieve effective enzymatic saccharification, it is necessary to assess the effect of differences in the structure of lignin on the adsorption of the enzyme onto lignin. Therefore, it is important to evaluate the effect of lignin in hardwood pulp on enzymatic saccharification. The enzymatic saccharification rate of hardwood SP was compared to that of softwood SP, as described below. In addition, the amount of enzyme adsorbed onto residual lignin in different types of pulp was estimated to determine the differences between softwood and hardwood SP in the rate of enzymatic saccharification.

The ultimate goal is to increase the rate of enzymatic saccharification for more effective ethanol production from SP. One feasible way to increase enzymatic hydrolysis is delignification of pulp. The decrease of lignin content in pulp would increase the enzymatic saccharification efficiency by decreasing the adsorption of enzyme to lignin in pulp.

The beating treatment, which is an established technology in the pulp and paper industry is expected to be another effective approach. The structure of the fiber is expected to be changed during beating because of the resulting fibrillation and fiber swelling, which increase the specific surface area of the fibers [10]. Several studies have reported that a large surface area of cellulose tends to increase the effectiveness of enzymatic hydrolysis [11, 12].

The objective of this study is to estimate the effect of residual lignin and pulp swelling on enzymatic saccharification. This information can then be used to increase the production of monosaccharides and ethanol from SP. First, we applied the Py-GC/MS method to quantification of the amount of enzyme adsorbed onto lignin in enzyme-treated softwood SP and hardwood SP. Second, we evaluated an increase in the water retention value as a measure of the specific surface area of pulp after various beating treatments. Third, the effect of delignification of SP on the rate of enzymatic saccharification was evaluated.

Experimental
Preparation of pulp samples
Larch (L. leptolepis) SP and Acacia (Acacia mearnsii) SP were prepared from oven-dried 45 g of chip under the following conditions: cooking temperature 135 ± 1 °C or 145 ± 1 °C, cooking time 0.5–4 h, SO₂ concentration 0.85 ± 0.2 mol/L, Mg(OH)₂ concentration 0.20 mol/L or 0.39 mol/L, pH 2.0 or 1.4, and liquid-to-wood (L/W) ratio 5 mL/g. Before the acid sulfite cooking, wood chips were treated with cooking liquor at 80 °C for 4 h.

Larch soda–AQ pulp samples with the kappa number 25.5 and 56.6 were prepared at 158 °C for 4.5 h, active alkali charge 28%, and the AQ dose 0.4% and 0.1%, respectively. Acacia Kraft pulp (KP) was prepared at 148 °C for 2.0 h, active alkali charge 23%, and sulfidity 25%.

The carbohydrate composition analysis of these pulp samples was conducted. The pulp sample (oven-dried, 300 mg) was first hydrolyzed with 72% sulfuric acid for 2.5 h and then further hydrolyzed with diluted 4% sulfuric acid at 121 °C for 1 h. Resulting five different
monosaccharides were determined using the ion chromato-
graphy system (Dionex ICS 3000 ion chromatograph,
Dionex, Sunnyvale, CA, USA). This system consisted of a 
single pump model (SP-1), a pulsed amperometric detector 
(PAD), an IonPac AS 7 column (Φ 4 mm × 250 mm), an 
IonPac AS 7 guard column (Φ 4 mm × 50 mm), and an 
auto sampler (AS). The chemical composition of the pulp 
samples is listed in Table 1.

**Enzymatic treatment of pulp**
An enzyme (GC220) was obtained from Genencore 
Kyowa Co., Ltd., Japan. GC220 is a mixture of cellulases
(endoglucanase, exoglucanase and β-glucosidase) 
produced from the mutant strains of *Trichoderma* (*T. reesei* 
and *T. longibrachiatum*). Fifty milligram of pulp was 
subjected to enzyme treatment with GC220 cellulase at 
the dose of 3.2 filter paper units (FPU) per gram of pulp 
and 12.7 FPU/g pulp at 45 °C, and the total volume was 
1.5 mL in 0.05 M acetate buffer (pH 4.5). The amount of 
glucose liberated from pulp was determined using an ion 
chromatograph (a Dionex ICS 3000 ion chromatograph).

For comparison of the amount of enzyme adsorbed 
onto lignin between larch SP and acacia SP, oven-dried 
0.5 g of pulp was suspended in 15 mL of 0.05 M acetate 
buffer (pH 4.5) at the dose of 12.7 FPU/g pulp, and was 
subjected to enzymatic treatment at 45 °C for 24 h.

In addition, the enzyme-treated pulp was soaked in 
0.05 M acetate buffer without enzyme for 20 min and 
then separated by filtration or centrifugation at 3000 g for 
5 min. This treatment was repeated 3 times. The sepa-
rate pulps were again soaked in distilled water for 20 min 
and then filtered. We expected that all of the enzyme 
adsorbed onto cellulose should be desorbed by this fil-
tration procedure. The amount of enzyme adsorbed 
onto lignin in the enzyme-treated pulp was determined 
by Py-GC/MS. After this treatment, the weight of the 
enzyme-treated pulp residue was measured. By sub-
tracting this weight from the original pulp weight before 
enzymatic treatment, the weight of soluble part by 
the treatment was calculated. The resolution ratio was 
declared as ratio of the weight of soluble part to the origi-
nal pulp weight.

The cellulase activity of the sample was measured 
globally according to the cellulase activity assay reported by 
the National Renewable Energy Laboratory (NREL) with 
minor modifications [13]. A value of 2.0 mg of a reducing 
sugar was calculated as glucose from 50 mg of Advantec 
No. 1 filter paper for 60 min. The minor modifications to 
the NREL method are as follows:

1. Glucose was quantified by ion chromatography 
   instead of a colorimetric assay with 3,5-dinitrosali-
cyclic acid (DNS).
2. The enzymatic reaction was stopped by heating at 
   100 °C for 5 min.
3. The enzymatic reaction was started by adding the 
   filter paper to the enzyme solution instead of add-
   ing enzyme to the filter paper saturated with buffer.

**Py-GC/MS analysis of enzyme-treated pulp**
The conditions for the Py-GC/MS analysis were as fol-
lows. Pyrolyzer, JHP-3 (Japan Analytical Industry Co., 
Ltd., Tokyo, Japan); pyrolysis temperature, 500 °C; pyrolysis

### Table 1 Chemical composition of pulp samples

| Cooking conditions |
|------------------|
| Yield (%) | Kappa number | Glucan (%) | Xylan (%) | Mannan (%) | Lignin (%) |
| Acacia SP a | 57.3 | 52.3 | 70.2 | 12.8 | 0 | 9.9 |
| Acacia SP b | 63.3 | 57.9 | 58.2 | 9.6 | 1.6 | 10.4 |
| Acacia SP c | 53.0 | 23.0 | 72.9 | 6.8 | 1.1 | 4.1 |
| Acacia KP | 56.0 | 22.8 | 67.1 | 13.0 | 1.1 | 3.0 |
| Larch SP d | 58.6 | 74.6 | 74.9 | 0 | 8.7 | 16.0 |
| Larch SP e | 74.8 | 127.0 | 55.8 | 0.9 | 16.0 | 22.8 |
| Larch SP f | 57.6 | 64.3 | 73.5 | 3.8 | 4.8 | 11.6 |
| Larch soda-AQ | 48.0 | 56.6 | 44.3 | 4.6 | 7.8 | 7.4 |
| Larch soda-AQ | 53.8 | 25.5 | 56.8 | 3.4 | 6.6 | 3.8 |

*Cooking conditions*

a pH 1.4, 145 °C for 0.5 h  
b pH 2.0, 135 °C for 4 h  
c pH 2.0, 145 °C for 3 h  
d pH 1.4, 145 °C for 1 h  
e pH 2.0, 145 °C for 1.5 h  
f pH 2.0, 145 °C for 2 h
time, 4 s; pyrolyzer temperature, 250 °C; transfer line temperature, 250 °C; GC/MS system, GCMS-QP 5050 A (Shimadzu Corporation, Kyoto, Japan); column, HP 1-MS (30 m × 0.25 mm; film thickness, 0.25 μm); column temperature, 50 °C for 1 min, raised to 280 °C at the rate of 5 °C/min, then maintained at 280 °C for 13 min; carrier gas, helium, injection temperature, 280 °C, interface temperature, 280 °C; ion source temperature, 210 °C, and ionization energy, 70 eV. The n-eicosane solution was used as an internal standard. The concentration of n-eicosane in ethyl acetate was 0.2 μg/μL.

To build the calibration curve for enzyme quantitation, 1–3 μL aliquots of GC220 diluted 20-, 50-, and 200-fold (0.3–6.5 mFPU) and 2 μL of n-eicosane solutions were added to filter paper and analyzed using Py-GC/MS. The two ion peaks of pyrolysis products, m/z 117 and 130 from the enzyme, which were identified as indole and methylindol, respectively, were used for enzyme quantitation. The enzyme-treated pulp samples were 150–200 μg for the Py-GC/MS analysis [6].

Estimation of lignin content in enzyme-treated pulp by means of Py-GC/MS

Guaiacol, 4-methylguaiacol, 4-vinylguaiacol, eugenol, vanillin, cis-isoegenol, 4-propylguaiacol, trans-isoegenol, acetoguaiacone, dihydroxy coniferyl alcohol, cis-coniferyl alcohol, and cis- and trans-coniferyl aldehydes were obtained as guaiacyl-type pyrolysis products from pulp. Syringol, 4-methylsyringol, 4-vinylsyringol, 4-allylsyringol, syringaldehyde, cis- and trans-propenylsyringol, propiosyringone, and trans-sinapyl alcohol were obtained as syringyl-type pyrolysis products. These pyrolysis products from lignin were used in the estimation of lignin content of pulp [14].

Untreated larch SP and acacia SP were analyzed by Py-GC/MS in order to determine the factor for lignin ($f_{lignin}$). The $f_{lignin}$ was determined using the following formula:

$$f_{lignin} = \frac{C_{lignin} \times 10^{-2} \times M_{sample}}{A_{IS}}$$

where $A_{IS}$ is the sum of the total ion chromatogram (TIC) peak areas of lignin pyrolysis products, $A_{IS}$ is the TIC peak area of the internal standard; $C_{lignin}$ is the lignin content (%) of the untreated pulp, $M_{sample}$ is the sample weight (g), and $M_{IS}$ is internal standard weight (ng). Lignin% content in the enzyme-treated pulp samples ($C_{lignin \ in \ residue}$) was estimated using the following formula:

$$C_{lignin \ in \ residue} (\%) = \frac{A_{lignin}}{A_{IS}} \times f_{lignin} \times M_{IS} \times 100/M_{sample}. \quad (2)$$

**Results and discussion**

**Enzymatic hydrolysis of larch pulp and acacia pulp**

Figure 1 shows the results of enzymatic treatment of larch and acacia pulp samples at an enzyme dose of 3.2 FPU/g pulp. In the case of larch pulp, the enzymatic saccharification rate of larch SP was higher than that of soda–AQ pulp. On the other hand, the behavior of enzymatic saccharification of acacia pulp samples was different from that of larch pulp samples. Acacia SP showed a lower enzymatic saccharification rate compared with
Acacia KP. Acacia SP might contain a factor contributing to the lowered enzymatic saccharification. It is well known that the residual lignin inhibits enzymatic saccharification of pulp because lignin ties up the enzyme to hydrolyze carbohydrates.

When the enzymatic saccharification was conducted at an enzyme dose of 12.7 FPU/g, the enzyme saccharification of acacia SP was almost the same as that of KP (Fig. 2). The increase of enzyme dose enhanced enzyme saccharification of acacia SP. Compared with the dose 3.2 FPU/g, the amount of glucose released from acacia SP treated with the enzyme at 12.7 FPU/g was close to that of filter paper. It is likely that at the high dose of the enzyme, the residual lignin in pulp has a relatively weak impact on the enzymatic saccharification rate because there is an excess of the enzyme.

Estimation of the amount of enzyme adsorbed onto lignin in enzyme-treated softwood SP and hardwood SP

The amount of enzyme adsorbed onto residual lignin in acacia SP and larch SP was estimated using Py-GC/MS in order to identify a cause of the difference in the enzymatic saccharification rate between the acacia and larch pulps. Figure 3 shows a pyrogram of untreated and enzyme-treated acacia SP. The two ion peaks of pyrolysis products from the enzyme, $m/z$ 117 and 130, were not detected in untreated acacia SP. On the other hand, these ion peaks were detected in enzyme-treated acacia SP. This result indicated that the amount of enzyme adsorbed onto acacia SP could be estimated using Py-GC/MS without any interference from other pyrolysis peaks of acacia SP. In addition, both syringyl- and guaiacyl-types of lignin pyrolysis products were obtained from acacia SP.

Figure 4 shows a calibration curve for quantification of GC220 (enzyme). There was a good correlation between the amount of GC220 (dose) and the sum of two peak areas. H'ng YY et al. [7] have reported that the correlation coefficients of the calibration curve between enzyme protein content calculated from elemental analysis of freeze-dried GC220 solution and the peak areas determined
by Py-GC/MS was 0.975 [7]. In this study, we estimated the enzyme amount as an enzyme unit (FPU), however similar calibration curve was obtained, and we confirmed that the amount of enzyme adsorbed in residual lignin can be detected by Py-GC/MS. Table 2 shows the results of Py-GC/MS analysis of enzyme-treated acacia SP and larch SP. The resolution ratio of acacia SP caused by the enzymatic treatment was 46.7%, which was lower compared to larch SP, even though the lignin content of acacia SP was lower than that of larch SP. The amount of enzyme adsorbed onto lignin in enzyme-treated acacia SP was estimated at 61 FPU/g lignin, which was higher compared to enzyme-treated larch SP (35 FPU/g lignin). We found that the residual lignin in acacia SP could more easily adsorb enzyme to hydrolyze carbohydrates compared to larch SP, and that it is likely to interfere with the binding of the enzyme to cellulose. Table 2 also shows the total sulfur content in untreated acacia SP and larch SP. The sulfur content of larch SP was higher than that of acacia SP. The higher sulfur content of pulp suggests that the residual lignin was highly sulfonated. Erikson et al. [18] reported that adding a negatively charged surfactant during the enzymatic hydrolysis decreases adsorption of the enzyme to steam-pretreated spruce. They explained this effect by electrostatic repulsion between similarly (negatively) charged enzyme and lignin surface (due to adsorption of the surfactant). The enzyme has a negative charge in the acetate buffer (pH 4.5) because of its isoelectric point (pI). For example, the pI of Cel7A, which is one of the most typical cellulases, is approximately 3.9 [18]. The sulfonated lignin also carries a negative charge.
and should prevent the adsorption of the enzyme because of electrostatic repulsion. The residual lignin in larch SP, which had more sulfonated lignin than did acacia SP, should be more effective in preventing the adsorption of the enzyme via electrostatic repulsion.

In addition, the lignin content (% on untreated pulp) of larch SP decreased from 16% to 8% during enzymatic hydrolysis as shown in Table 2. A similar result was reported by Takahashi and Ohi [6]. It has been reported that the lignin content of larch SP with the kappa number 127 after 48 h of enzymatic treatment with enzyme at 14.4 FPU/g pulp was half that of the untreated SP. The residual lignin of larch SP was sulfonated, and the sulfonated lignin should be dissolved during enzymatic treatment. On the other hand, the lignin content of acacia SP did not change much during enzymatic hydrolysis, even though the residual lignin was sulfonated.

Some authors formulated possible requirements for removal of sulfonated lignin from wood during cooking: (1) decomposition of lignin into smaller molecules, and (2) cleavage of the link between lignin and other chemical components of wood (e.g., hemicellulose) [19]. It is thought that the residual lignin in acacia SP has either high molecular weight or linkage with hemicellulose, and sulfonated lignin is hard to liberate from acacia SP during enzymatic saccharification.

**Table 2** The amount of enzyme adsorbed onto acacia and larch SP estimated using Py-GC/MS analysis and sulfur content of untreated SP

|                      | Acacia SP | Larch SP |
|----------------------|-----------|----------|
| Kappa number         | 52.3      | 74.6     |
| Lignin content (%)   | 9.9       | 16.0     |
| Resolution ratio of pulp after enzymatic treatment (%) | 46.7 | 69.3 |
| Lignin content (%) on residue | 17 | 27 |
| (% on untreated pulp) | 9 | 8 |
| The amount of enzyme adsorbed onto pulp (FPU/g residue) | 10 | 9 |
| (FPU/g untreated pulp) | 6 | 3 |
| The amount of enzyme adsorbed onto lignin (FPU/g lignin in residue) | 61 | 35 |
| Sulfur content (μmol/g pulp) | 79.4 | 164 |
| (μmol/g lignin) | 802 | 1020 |

Enzymatic treatment: pulp 0.5 g, acetate buffer 0.05 M, pH 4.5 and 15 mL and temperature at 45 °C

**Table 3** The water retention value of pulp samples

|                      | Untreated 2500 PFI revolutions | Untreated 5000 PFI revolutions | Untreated 7500 PFI revolutions |
|----------------------|-------------------------------|-------------------------------|-------------------------------|
| Acacia SP            | 158                           | 188                           | 191                           |
| Larch SP             | 165                           | 254                           | 260                           |
| Filter paper         | 87                            | –                             | –                             |

Figure 5 shows enzymatic treatment of beaten acacia SP and larch SP, with the enzyme dose at 3.2 FPU/g pulp. The amount of glucose released from unbeaten filter paper at 24 h was 14.9 mg, and it increased to 18.9 mg after beating at 7500 PFI revolutions. The amount of glucose liberated from unbeaten larch SP and unbeaten acacia SP at 24 h was 11.7 mg and 6.8 mg, respectively. When larch SP was beaten at 7500 revolutions, the amount of glucose released from larch SP increased to 18.9 mg, which was almost identical to that of beaten filter paper. The amount of glucose released from beaten acacia SP at 7500 PFI revolutions was slightly increased, but it was still lower than that of unbeaten filter paper.

Mooney et al. [20] compared the cellulose hydrolysis conversion between sulfonated Douglas fir refiner mechanical pulp (RMP) and untreated RMP. The sulfonated RMP with sodium sulfite exhibits increased fiber swelling compared to untreated RMP. They found that the cellulose conversion of sulfonated RMP was higher than that of untreated RMP, even though the lignin content of sulfonated RMP was high. Nonetheless, delignified RMP with lower lignin content showed better cellulose hydrolysis conversion than did sulfonated RMP. Those authors offered a possible explanation: the high lignin content might restrict the access of the enzyme to...
cellulose, even though RMP exhibits greater fiber swelling [20]. In the present study, even though acacia SP had lower lignin content compared to larch SP, the increase of fiber swelling did not affect the rate of enzymatic saccharification of acacia SP significantly. The residual lignin in acacia SP should strongly inhibit the enzyme saccharification by adsorbing enzyme to lignin, compared with larch SP (Table 2), and this effect should attenuate the enhancement of enzymatic saccharification rate by beating. We found that the effect of pulp swelling on the increase of enzymatic saccharification was small, whereas the residual lignin in pulp strongly interferes with the adsorption of the enzyme to cellulose.

Figure 6 shows the results of enzymatic treatment with the enzyme dose at 12.7 FPU/g. a larch SP with the kappa number 74.6 and b acacia SP with the kappa number 52.3. Solid diamond, solid circle, solid square and solid triangle: the number of PFI revolutions 0, 2500, 5000, and 7500, respectively, and open diamond, open triangle: filter paper, PFI revolutions 0 and 7500, respectively. Enzymatic treatment: pulp 50 mg, acetate buffer pH 4.5 and 1.5 mL and temperature at 45 °C.
of enzymatic saccharification rate of acacia SP induced by the beating was higher compared to the 3.2 FPU/g dose. As shown in Fig. 2, the high enzyme dose (12.7 FPU/g) attenuated the inhibitory effect of residual lignin in pulp on the enzymatic saccharification. This is one of the reasons why pulp swelling induced by beating effectively increased the enzymatic hydrolysis of acacia SP when the hydrolysis was conducted at a high dose of the enzyme.

Enhanced enzymatic saccharification of larch and acacia SP after delignification

Figure 7 shows comparison of the rate of enzymatic saccharification between delignified SP and undelignified SP. The chemical composition of delignified SP is listed in Table 4. Delignified larch and acacia SP showed a higher enzymatic saccharification rate than did undelignified pulp samples. The rate of enzymatic saccharification also tends to increase when the kappa number of acacia SP and larch SP decreased. The delignification process was effective at increasing the enzymatic saccharification of acacia SP and larch SP.

The enzymatic saccharification rate of delignified larch SP was higher than that of filter paper and delignified acacia SP. Acacia SP showed similar enzymatic saccharification rate compared to filter paper. The water retention value of delignified larch SP was higher than that of filter paper and delignified acacia SP (Table 4), and this effect is expected to increase the rate of enzymatic saccharification of delignified larch SP.

The delignification treatment significantly increased the enzymatic saccharification rate of acacia SP (Fig. 7), but that of acacia SP was only slightly increased by beating, with the enzyme dose at 3.2 FPU/g (Fig. 5). In addition, the increase of the kappa number decreased the enzymatic saccharification of SP. This observation suggests that the residual lignin in SP is the main inhibitor of enzymatic saccharification. Thus, delignification should be kept in mind as a high-priority technique for enhancing enzymatic saccharification of SP. In addition, we hypothesized that delignification of SP followed by the beating treatment should have a synergistic effect on enzymatic saccharification of SP. This is because Fig. 5 shows that the residual lignin in acacia SP attenuated the increase of enzymatic saccharification rate after the beating treatment. The delignification process decreases this inhibition and is expected to enhance the increase of enzymatic saccharification by beating, at least additively.
Conclusions

First, we applied the Py-GC/MS method to the quantification of the enzyme adsorbed onto lignin in enzyme-treated softwood SP and enzyme-treated hardwood SP. The resolution ratio of acacia SP after the enzymatic treatment was lower compared to larch SP even though lignin content of acacia SP was lower. The amount of enzyme adsorbed onto residual lignin in enzyme-treated acacia SP (as estimated by Py-GC/MS) was 61 FPU per 1 g of lignin, which was higher compared to enzyme-treated larch SP (35 FPU/g lignin). We found that the residual lignin in acacia SP could more easily adsorb enzyme to hydrolyze carbohydrates, compared to larch SP, and we theorized that residual lignin is likely to interfere with the binding of the enzyme to cellulose.

Second, the effect of beating of pulp on the rate of enzymatic saccharification was assessed. The increase of glucose release from larch SP after beating was higher compared to acacia SP. The pulp swelling was increased by beating and the swelling efficiently increased the enzymatic saccharification rate, but only when the residual lignin in pulp could not interfere with the adsorption of the enzyme to cellulose.

Third, we studied the effect of delignification of SP on the rate of enzymatic saccharification. The delignification treatment was more effective than the beating treatment at enhancing enzymatic saccharification of both acacia SP and larch SP. The delignification process should be considered a high-priority technique for enhancing enzymatic saccharification of SP.

Abbreviations
AQ: Anthraquinone; CBD: Cellulose-binding domain; FPU: Filter paper units; KP: Kraft pulp; IS: Internal standard; Py-GC/MS: Pyrolysis-gas chromatography/ mass spectrometry; RMP: Refiner mechanical pulp; SP: Acid sulfite pulp; SSL: Spent sulfite liquor; TIC: Total ion chromatogram.

Acknowledgements
This work was partly supported by the Sasakawa Scientific Research Grant from The Japan Science Society. The larch soda–AQ pulps and acacia KPs were kindly provided by Dr. Shiho Takahashi in Forestry and Forest Products Research Institute.

Authors’ contributions
KT had the major role in implementing the overall work including the experimental design, performing the required experiments and writing the manuscript. ANI was a major contributor in “Py‑GC/MS analysis” part, assisting experimental design, performing the required experiments and writing the manuscript. KT had the major role in implementing the overall work including the technical management of the research. KO had the major role in writing this manuscript. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Competing interests
The authors declare that they have no competing interests.

Received: 24 April 2020 Accepted: 7 September 2020
Published online: 21 September 2020

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