Todaka *et al. J Wood Sci* (2019) 65:49
https://doi.org/10.1186/s10086-019-1829-x

Journal of Wood Science

ORIGINAL ARTICLE

Open Access

Delignification of softwood by glycerol from biodiesel by-product I: model reaction using glycerol and fatty acid sodium soap mixture for pretreatment on bioethanol production

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Abstract

This study investigated the use of glycerol–fatty acid sodium soap mixtures to delignify woody biomass as a model for utilization of crude glycerol by-product from biodiesel manufacture. Lignin-bearing glycerol was also produced. Delignification was carried out using glycerol mixed with sodium salts of laurate, stearate, oleate, or linoleate at 100–250 °C for 0.5–3 h. Oak, beech, bamboo, and rice straw were easily delignified by 20% sodium oleate dissolved in glycerol at around 150 °C for 1 h. For softwood (Japanese cedar and spruce), delignification did not occur below 200 °C. However, the lignin content decreased from 37.5% in untreated Japanese cedar to 10.6% and from 29.4% in untreated spruce to 11.2% by treatment at 250 °C. Japanese cedar was not delignified in glycerol mixtures with oleic acid or sodium acetate. It is suggested that the surfactant activity of soap assists delignification, but the alkaline action of sodium acetate was not effective. Enzymatic saccharification of delignified Japanese cedar (9.3% lignin) and oak (3.6% lignin) samples gave glucose yields of 0.55–0.67 g/g after 72 h and these yields were comparable with that from pure cellulose (0.77 g/g). Lignin dissolution also increased the calorific value of the collected glycerol fraction from 20 to 25 MJ/kg. The results suggest that a waste-free delignification method can be achieved based on the combined processes of biodiesel and bioethanol production.

Keywords: Delignification, Biodiesel by-product glycerol, Sodium soap, Lignocellulose, Softwood

Introduction

Glycerol is a useful material in a range of industries including food and beverage manufacture, pharmaceuticals, cosmetics, and personal care products [1, 2]. However, the applications of glycerol in these industries are limited to the use of pure glycerol, such that the use of crude glycerol is not feasible because of its impure content. About 10% crude glycerol is produced from vegetable oil during biodiesel production. It contains impurities, including fatty acids and sodium soaps that are derived from the alkali catalyst used in general production process, such as NaOH or KOH [3]. The soap content in crude glycerol has been reported to range from 20 to 31% (w/w). As a by-product, the supply of glycerol is largely independent of its demand, with global production of glycerol from biodiesel production exceeding 2 million tons in 2012 [4]. New ways of using glycerol are therefore required to improve resource efficiency. For example, bioconversion technologies using fungi have been reported wherein glycerol is converted to bioproducts such as hydrogen, *n*-butanol, citric acid, and biopolymers [5]. Thus, the use of glycerol produced as a by-product of biodiesel could have important implications in the biorefinery industry.

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In bioethanol production, pretreatment to remove lignin (delignification) is an essential step that promotes enzymatic saccharification. Hence, many researchers have proposed various methods of delignification [6]. Physical and chemical treatment of woody biomass using glycerol from biodiesel production has been investigated as an alternative to other pretreatment methods, such as alkali or acid treatment, organosolv extraction, and ionic liquid extraction, for the production of bioethanol from lignocellulosic biomass [7]. For example, organosolv delignification has been carried out with 40–70% ethanol and an acid catalyst [8]. However, the low boiling point of the solvent meant that the delignification process required special equipment to achieve elevated pressure for the extraction. To avoid this, extraction with glycerol, which has a boiling point of 290 °C, has been investigated as a method for atmospheric organosolv delignification. This approach has the potential to offer cost savings to both biodiesel and bioethanol production if the glycerol by-product of biodiesel could be used.

An example of delignification using glycerol and sodium methoxide (CH_3ONa), a catalyst used in biodiesel production, has been reported [9]. Those results showed that approximately 80% of the lignin in sugarcane bagasse was removed by treatment with 0.87% CH_3ONa at 149 °C for 1.4 h. Moreover, considerable research effort has focused on organosolv delignification using glycerol with and without catalysts for the treatment of wheat straw and sugarcane bagasse [10–13]. However, most of the works in this area have investigated lignocellulosic materials that are relatively easy to delignify, such as bagasse and straw.

Lignin polymer consists of guaiacyl units (G lignin) derived from coniferyl alcohol, syringyl units (S lignin) derived from sinapyl alcohol, and *p*-hydroxyphenyl units (H lignin) derived from *p*-coumaryl alcohol [14]. Softwood lignin consists mainly of G lignin. As is well known, the G lignin polymer is more difficult to break down to its low molecular weight constituents by chemical treatment than the mixed G–S–H lignin polymer because it is rich in condensed linkage. In addition, the structural arrangements of softwood and hardwood lignins are also important considerations [15]. For hardwoods, lignin and hemicellulose are arranged in parallel in the cell walls, but in softwoods the subcomponents are arranged in a complex three-dimensional structure. This makes it more difficult to remove lignin from softwood.

According to our preliminary experiments using CH_3ONa [9], the lignin content of bamboo and rice straw decreased from 28 to 15% and from 26 to 14% (w/w), respectively. However, Japanese cedar (*Cryptomeria japonica*) was not delignified, which demonstrated the difficulty of delignifying softwood. Delignification of Japanese cedar (a proxy for all softwoods) has, however, been reported. This involved microwave-assisted pretreatment and the use of aqueous glycerol with acid catalysts of different pK_a (e.g., acetic, citric, lactic, maleic, or malonic acids, H_3PO_4, HCl, or H_2SO_4) [16]. Delignification by organosolvolysis seemed to be strongly correlated with the pK_a of the catalysts used, if not perfectly. In this way, it is considered that delignification of Japanese cedar could be achieved based on a doped glycerol solvent system.

The aim of the present study was to develop a new method to utilize crude glycerol that is generated as a by-product of biodiesel production. In particular, our focus was on the impacts of the amount and type of fatty acid salt (sodium soap) used with the glycerol and the effects on delignification. This delignification method was investigated as a pretreatment of biomass in bioethanol production. For comparison, the process was carried out with softwood (Japanese cedar and spruce), hardwood (oak and beech), and gramineous plants (bamboo and rice straw). Enzymatic saccharification was carried out for treated biomass samples to confirm the effects of delignification. We also assessed the potential use of the resultant lignin-containing glycerol as a fuel by evaluating its physical properties, such as the higher heating value (HHV), lower heating value (LHV), and viscosity. From these experiments, a waste-free delignification method for biodiesel and bioethanol production has been proposed. The delignification method described in this study has been submitted as a patent in Japan [17].

Materials and methods

Materials

Samples of Japanese cedar (*Cryptomeria japonica*), spruce (*Picea sitchensis*), oak (*Quercus crispula*), beech (*Fagus crenata*), bamboo, and rice straw were selected for different types of lignocellulosic biomass. Planer dust of Japanese cedar was obtained from the Miyazaki Prefectural Forestry Technology Center (Misato town, Japan). Bamboo powder was obtained from the Fukuoka Industrial Technology Center in Okawa, Fukuoka Prefecture. Oak was purchased from Kose (Fukui City, Japan). Rice straw was obtained from a rice field in Kushima City, Miyazaki Prefecture. Spruce and beech were purchased from a sawmill in Fukuoka Prefecture. The samples were sieved to give a size distribution of 177–350 μm (42–80 mesh).

A model of crude glycerol was made by mixing glycerol and a fatty acid sodium salt such as sodium laurate, sodium stearate, sodium oleate (all from Fujifilm Wako Pure Chemical, Osaka, Japan), or sodium linoleate (Tokyo Chemical Industry, Tokyo, Japan). Sodium acetate and oleic acid (both from Fujifilm Wako Pure Chemical) were also used to investigate the impact of carbon chain length and alkalinity. Abbreviations of the sodium salts and fatty acids used are shown in Table 1.

Delignification by glycerol with sodium soaps

Delignification of wood powder was conducted in a 1-L three-neck flask that was fitted with a mechanical stirrer. Crude glycerol (100 g) that contained 0–20% (w/w) sodium soap was added to the three-neck flask before adding a 10% loading of wood powder (~ 10 g). The treatments were conducted at 100–250 °C with exposure times ranging from 0.5 to 3 h under atmospheric pressure without reflux.

After each reaction, the mixture was cooled to 70–80 °C before the contents of the three-neck flask were transferred to a 106-μm sieve (Φ 75 × 20 mm) mounted above a receiver. This entire module was then transferred to a centrifugation tube (Φ 98 × 100 mm) and centrifuged at 1870×g (3000 rpm) for 5 min (Himac SCT5BC, Hitachi Koki, Tokyo Japan). The treated wood sample (solid) and glycerol fraction (liquid) were retained in the sieve and receiver, respectively. The solid fraction was washed three times with water and then dried at 105 °C.

Determination of chemical composition in treated biomasses

To understand the chemical composition of the treated biomass, the contents of lignin, α-cellulose, and alkali-soluble cellulose were determined according to standard procedures.

The lignin content of the treated and untreated wood powders was determined by the Klason method, which is widely accepted for lignin determination [18, 19]. A portion of dried sample (1 g) was immersed in 15 mL of 72% H_2SO_4 (w/w) for 4 h at room temperature with regular stirring. The sample was then diluted to 3% H_2SO_4 (w/w) by adding 560 mL of water. Hydrolysis was then achieved using an autoclave (SD-30 N, Tomy Seiko, Tokyo, Japan) operated at 121 °C and 0.1 MPa gauge pressure for 30 min. The contents were filtered using a sintered-glass filter (1G4, AGC Techno Glass, Shizuoka, Japan) and dried at 105 °C until a constant mass was achieved. The constant mass was compared with the original mass to yield the lignin content.

Holocellulose, which consists of α-cellulose and alkali-soluble cellulose, in all biomasses was determined by the Wise method using $NaClO_2$ [20]. A portion of dried sample (2.5 g) was immersed in 100 mL of ion-exchanged water. Then 1.0 g of $NaClO_2$ and 0.2 mL of acetic acid were added. The mixture was heated at about 80 °C for 1 h and this cycle was repeated four times. The contents were filtered using a sintered-glass filter (1GP100) and washed with acetone and water. Finally, the residue was dried at 105 °C until a constant mass was achieved. This was compared with the original mass to yield the holocellulose content. A portion of dried holocellulose (1 g) was immersed in 25 mL of 17.5% NaOH solution to separate the hemicellulose (i.e., alkali-soluble cellulose). After 30 min, ion-exchanged water was added and the gel-like mixture was centrifuged at 1210×g (3000 rpm) for 5 min before filtration on a sintered-glass filter (1GP250). The material on the filter was neutralized washed with 40 mL of 10% acetic acid and ion-exchanged water, respectively. Then, the sample was dried at 105 °C until a constant mass was achieved. This mass was compared with the original mass to give the α-cellulose content. The filtrate liquid (i.e., 17.5% NaOH-soluble fraction of wood powder treated with 20% $C_{18:1}Na$ in glycerol, 250 °C, 1 h) was collected. The soluble material in the 17.5% NaOH solution was precipitated using 30% acetic acid. The NaOH-soluble component was filtered and air dried. The contents of xylose and glucose were measured by high-performance liquid chromatography (HPLC) after acid hydrolysis of the 17.5% NaOH-soluble component as per the Klason method. The HPLC conditions are described in "Enzymatic saccharification" section.

Table 1 Fatty acid sodium soaps used in this study and their abbreviations

Fatty acid sodium salt	Abbreviation
Sodium acetate	C_2Na
Sodium laurate	$C_{12}Na$
Sodium stearate	$C_{18:0}Na$
Sodium oleate	$C_{18:1}Na$
Sodium linoleate	$C_{18:2}Na$
Oleic acid	$C_{18:1}$

X-ray diffraction

The polycrystalline nature of treated or untreated Japanese cedar was measured by X-ray diffraction (XRD; LabX XRD-6100, Shimadzu, Kyoto, Japan). The measurements were recorded using a wavelength of 1.5405 nm (CuKα), a 2θ drive axis, a scan range of 5–30°, and a scan speed of 2°/min. The degree of crystallinity was calculated from the crystal peak and amorphous broad areas.

Enzymatic saccharification

Saccharification used 1 g of substrate and 0.5 g of cellulase (AC 40, HBI Enzyme, Hyogo, Japan) in 100 mL of citric acid buffer solution at pH 4.5. The reaction proceeded for 72 h with the sample incubated at 50 °C with stirring at 1000 rpm. The amount of glucose produced was determined by high-performance liquid chromatography (HPLC). The HPLC system (LC Net-II/ADC, Jasco, Tokyo Japan) consisted of a refractive index (RI) detector (RI-2031 Plus, Jasco), a HPLC pump (PU-970, Jasco), a three-line degasser (DG-980-50, Jasco), a column oven (CO-965, Jasco), and an NH_2 column (Asahipak NH2P-50G; 5 μm, 4.6 × 250 mm; Showa Denko, Tokyo Japan) connected with a guard column (NH2P-50G 4A, Showa Denko). The oven temperature was set at 40 °C. The mobile phase consisted of 75% acetonitrile (Wako first grade, Wako Pure Chemical Industries) and 25% ion-exchanged water; its flow rate was fixed at 1 mL/min. The total analysis time was 20 min. The glucose yield (g/g) was calculated as the amount of glucose produced per gram of substrate.

Fuel properties of glycerol fraction

The higher heating value (HHV) of the recovered glycerol fraction was measured with a bomb calorimeter (C5003, IKA-Works, Staufen im Breisgau, Germany). The lower heating value (LHV) was calculated from the CHN(O) composition obtained using an elemental analyzer (2400-II, Perkin Elmer, Waltham, MA, USA). The viscosity of the glycerol fraction was measured using a rheometer (DV-III, Brookfield, Toronto, Canada) to evaluate its potential for use as a liquid fuel.

Results and discussion

Effect of amount of $C_{18:1}$Na on delignification of Japanese cedar

Delignification of Japanese cedar was investigated using varying amounts of $C_{18:1}$Na in glycerol at 250 °C for 0.5 to 3 h. Figure 1 shows the lignin content of Japanese cedar treated under different conditions. The lignin content of untreated Japanese cedar was 37.5% (w/w). When only glycerol or 5% $C_{18:1}$Na was used, no delignification occurred after 3 h of reaction. Conversely, the use of 10 or 20% $C_{18:1}$Na in glycerol promoted the delignification of Japanese cedar, and the lignin content decreased from 37.5 to 16.3 and 10.6%, respectively, after 3 h of reaction. Delignification proceeded rapidly during the first hour of reaction, after which the reaction rate appeared to slow. At 230 °C, the lignin content decreased to 19.2%. The use of 20% $C_{18:1}$ (not the sodium salt) in glycerol at 250 °C for 3 h decreased the lignin content to 30.7%. These results suggested that the present delignification process may require surfactant activity or alkalinity offered by the salt. It is possible that glycerol can more easily penetrate the complex binding of softwood subcomponents because of the salt surfactant activity. In addition, it likely that decomposition products derived from lignin can be dispersed in glycerol by the surfactant activity of the sodium soap. However, full explanation of these phenomena will require further detailed research to elucidate the mechanisms of delignification. Under the conditions examined in this study, delignification of Japanese cedar was most successful using 20% $C_{18:1}$Na in glycerol at 250 °C for 1 h.

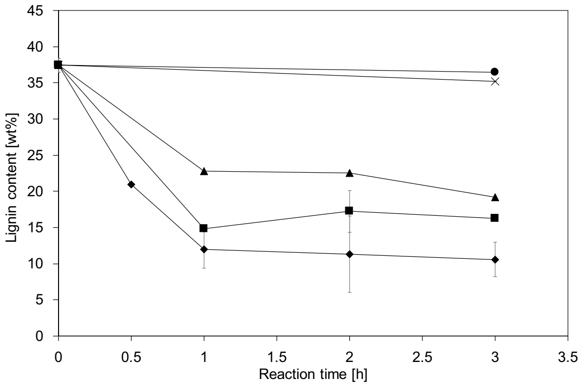

Fig. 1 Change in lignin content of Japanese cedar with treatment time for different concentrations of sodium oleate or sodium acetate in glycerol at different temperatures. Error bars shows standard deviation ($n = 3$). Symbols: times, glycerol only; filled circle, 5% $C_{18:1}$Na at 250 °C; filled square, 10% $C_{18:1}$Na at 250 °C; filled rhombus, 20% $C_{18:1}$Na at 250 °C; filled traingle, 20% $C_{18:1}$Na at 230 °C. Fatty acid sodium soaps are defined in Table 1

Effect of different sodium soaps on delignification

To compare the effects of surfactant activity and alkalinity on delignification, oak was treated with 20% (w/w) solutions of C_2Na and $C_{18:1}$Na in glycerol, respectively, at 250 °C for 1 h. Japanese cedar was subjected to the same treatments, but treatments with 20% solutions of C_{12}Na, $C_{18:0}$Na, and $C_{18:2}$Na in glycerol were also performed to investigate the impact of using saturated and unsaturated sodium soaps on delignification (see Fig. 2). The lignin contents of Japanese cedar treated with C_2Na, C_{12}Na, $C_{18:0}$Na, $C_{18:1}$Na, and $C_{18:2}$Na were 31.7, 11.5, 15.5, 9.4, and 10.7%, respectively. Comparison of the results for different surfactant carbon length (C_2Na, C_{12}Na, and $C_{18:0}$Na) showed the effectiveness of the longer chain lengths (12–18) for delignification of Japanese cedar, while C_2Na was relatively ineffective. Although the C_2Na solution is alkaline, sodium acetate has no surfactant activity. This suggests that the delignification process requires both alkalinity and surfactant activity. The results for $C_{18:0}$Na, $C_{18:1}$Na, and $C_{18:2}$Na showed that all of the sodium salts promoted delignification, suggesting that the number of double bonds in the sodium soap has little or no effect on the delignification efficiency.

The results for the delignification of oak are also shown in Fig. 2. The lignin content decreased from 26.4% for untreated oak to 8.6 and 3.6% following treatment at 250 °C for 1 h with 20% solutions of C_2Na and $C_{18:1}$Na, respectively. Thus, for a hardwood like oak, delignification was easily accomplished, even with the use of C_2Na.

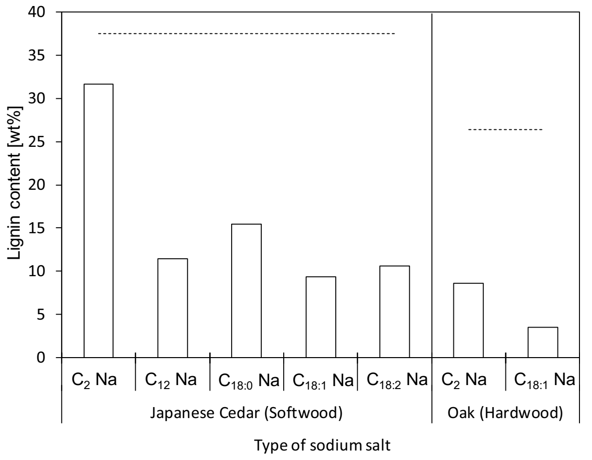

Fig. 2 Lignin content of Japanese cedar and oak after treatment with 20% sodium soaps dissolved in glycerol at 250 °C for 1 h. Dashed lines show lignin content of untreated samples

Effect of reaction temperature on delignification of different biomasses

Figure 3 shows the temperature dependence of delignification of various biomasses treated with 20% sodium oleate in glycerol for 1 h. The lignin contents of untreated Japanese cedar, spruce, oak, beech, bamboo, and rice straw were 38.2, 29.4, 21.3, 24.5, 25.8, and 26.3%, respectively. For the hardwoods (oak and beech), delignification occurred above 175 °C, with the lignin content of oak decreasing from 21.3 to 3.4%, and that of beech decreasing from 24.5 to 2.2%. For the gramineous plants (bamboo and rice straw), delignification occurred above 150 °C. The lignin content of bamboo decreased from 26.3 to 2.36%, and that of rice straw decreased from 25.8 to 10.8%. Thus, this delignification method using 20% sodium oleate in glycerol was suitable for hardwood and gramineous plants. For softwood (Japanese cedar and spruce), delignification was achieved at higher temperatures (over 200 °C), which was likely a result of the different structural arrangement of cell walls [15] and different lignin type for softwood species [21]. The results show that for delignification by glycerol with sodium soap, the character of the lignocellulosic biomass influences the temperature requirements of the treatment.

Figure 4 shows the changes in the chemical compositions of samples after treatment by glycerol with soap at 250 °C for 1 h. The solids' recovery after glycerol treatment at 250 °C ranged from 33.8 to 48.6% for all samples. The content of holocellulose in the treated samples decreased from raw samples and was comparable with the content of α-cellulose in each raw sample. This suggests that in addition to lignin removal, the treatment also caused thermal decomposition of hemicellulose. However, other causes are also conceivable; that is, α-cellulose was converted to be soluble in 17.5% NaOH. Table 2 shows xylose and glucose contents in the alkali-soluble (17.5% NaOH) fraction of each treated biomass. For the softwoods (Japanese cedar and spruce), glucose content was much higher than xylose in the alkali-soluble fraction. It was considered that some of the glucose was derived from α-cellulose. For the hardwoods and gramineous plants, the content of xylose was higher than that of glucose. It was considered that the alkali-soluble fraction was derived from hemicellulose, but from these results, it was also considered that part of the α-cellulose was converted to alkali-soluble cellulose. The extent of this phenomenon probably depends on the biomass type.

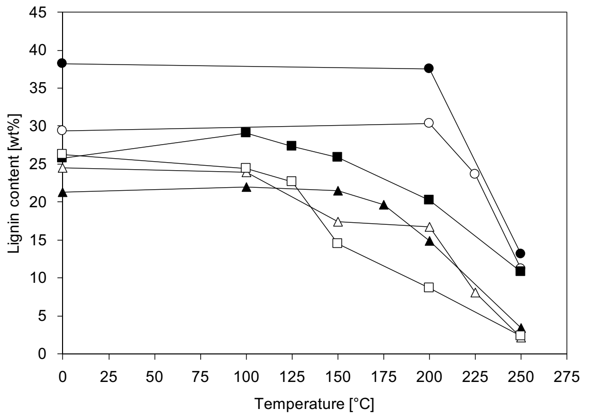

Fig. 3 Temperature dependence of delignification for different biomasses with 20% sodium oleate in glycerol. Symbols: filled circle, Japanese cedar; open circle, spruce; filled triangle, oak; open triangle, beech; closed square, rice straw; open square, bamboo. Data at 0 °C show lignin content of raw biomass

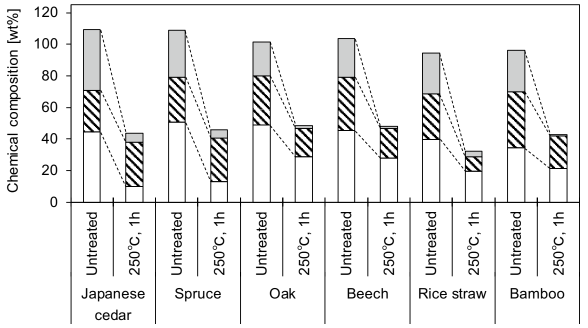

Fig. 4 Changes in chemical composition of different biomasses treated with 20% sodium oleate in glycerol at 250 °C for 1 h. White area, α-cellulose; hatched area, hemicellulose and alkali-soluble cellulose; gray areas, Klason lignin

Table 2 Xylose and glucose content in 17.5% NaOH-soluble fraction of biomass powder after treatment with 20% $C_{18:1}Na$ in glycerol at 250 °C for 1 h

	Japanese cedar		Spruce		Oak		Beech		Rice straw		Bamboo	
	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD
Xylose	9.7	2.9	7.8	0.5	57.1	1.1	63.9	5.0	69.7	5.3	60.2	4.5
Glucose	83.0	1.4	82.6	1.8	37.3	2.4	35.6	0.2	31.3	1.6	42.5	1.0

SD standard deviation; *n* = 3

Polycrystalline structure and degree of crystallinity of treated Japanese cedar

The XRD pattern of the cellulose type I polycrystalline structure shows three main peaks at 14.8, 16.8, and 22.6° [22]. Figure 5 shows the XRD results for untreated and treated Japanese cedar. The crystalline structure of Japanese cedar treated with glycerol for 3 h, and with 20% $C_{18:1}Na$ in glycerol for 0.5 h are cellulose I. However, the result for Japanese cedar treated with 20% $C_{18:1}Na$ in glycerol for 3 h showed only two peaks at 15.6 and 22.5°, which corresponds with cellulose IV peaks [22]. This result suggests that as delignification proceeds, the crystal structure changes from cellulose I to IV. However, curve fitting of cellulose IV and the measurement chart of Japanese cedar treated with 20% $C_{18:1}Na$ in glycerol at 250 °C showed differences in peak width. It was possible that the treated Japanese cedar contained both cellulose I and cellulose IV.

Table 3 shows the degree of crystallinity for Japanese cedar with and without treatment, and that of cellulose powder. The degree of crystallinity of Japanese cedar increased with delignification progress because of the removal of amorphous materials such as lignin and hemicellulose. This phenomenon has been reported previously [23]. The degree of crystallinity for Japanese cedar treated with 20% $C_{18:1}Na$ in glycerol at 250 °C for 3 h was close to that of cellulose powder. However, the crystallinity was lower than that of cellulose because of the increase in the alkali-soluble fraction. These results confirmed that delignification with glycerol and sodium soap increases the degree of crystallinity of the lignocellulosic substrate and changes the cellulose crystal structure.

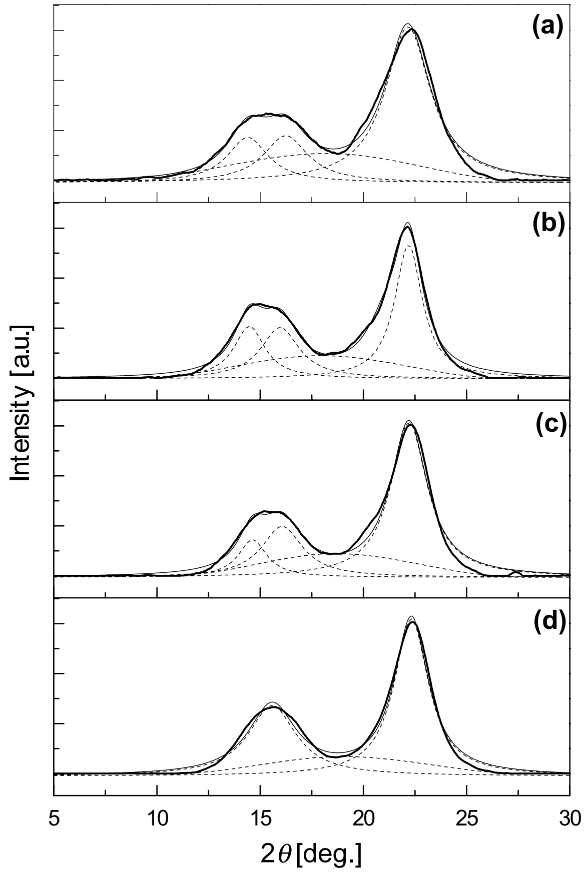

Fig. 5 X-ray diffraction patterns of **a** untreated Japanese cedar, and Japanese cedar treated with **b** glycerol at 250 °C for 3 h, **c** 20% sodium oleate in glycerol at 250 °C for 0.5 h, and **d** 20% sodium oleate in glycerol at 250 °C for 3 h. Bold solid line shows the X-ray diffraction pattern. Broken and solid lines show the peak separation and sum of the peaks, respectively

Enzymatic saccharification of collected solid fraction

Figure 6 shows the glucose yield produced by enzymatic saccharification from delignified Japanese cedar and oak. The run conditions are shown in Table 4. Cellulose powder was also enzymatically saccharified for reference. The glucose yield from cellulose powder (Run 1) was 0.75 g/g after 72 h. The untreated samples (Japanese cedar, Run 2; and oak, Run 8) showed no saccharification after 72 h. The glucose yields from Japanese cedar treated with glycerol and sodium soap (Runs 3–5) were 0.55–0.67 g/g. These results show that the delignification process was an effective pretreatment for enzymatic saccharification. However, Japanese cedar treated with only glycerol or C_2Na dissolved in glycerol (Runs 6 and 7) was not saccharified because Japanese cedar was not delignified. For oak, treatment in 20% solutions of $C_{18:1}Na$ (Run 9)

Table 3 Polycrystalline structure and degree of crystallinity of treated Japanese cedar

Japanese cedar	Polycrystalline type	Degree of crystallinity [%]
Untreated	I	73
Only glycerol 3 h	I	74
$C_{18:1}$ Na 20wt%, 0.5 h	I	77
$C_{18:1}$ Na 20wt%, 3 h	IV	80
Untreated cellulose powder	I	84

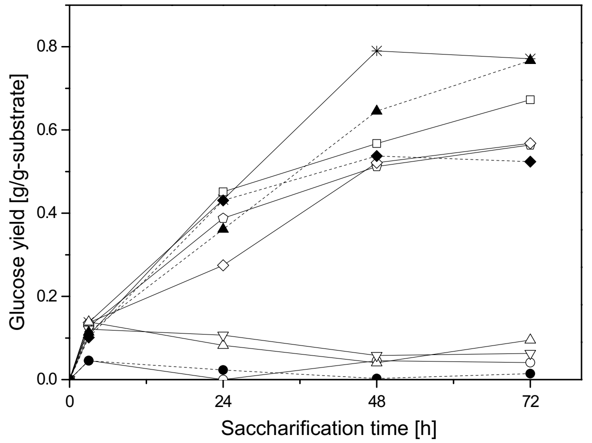

Fig. 6 Glucose yield by enzymatic saccharification from 1 g of Japanese cedar and oak powders treated with different conditions. Run conditions are shown in Table 4

and C_2Na (Run 10) in glycerol promoted enzymatic saccharification. These results were attributed to the decrease in lignin content and the change in the polycrystalline structure, both of which are beneficial for enzymatic saccharification.

Fuel characteristics of glycerol fraction-dissolved wood components

Generally, fuels with high calorific values are preferred. The glycerol fractions used in this study are expected to have high calorific values because the dissolved lignin and hemicellulose provide a high carbon content in the glycerol fraction. Therefore, to increase the calorific value of glycerol for it to be used as a fuel, solvolysis pulping experiments were performed repeatedly and the glycerol fraction was reused for pulping at 250 °C for 3 h (with 20% $C_{18:1}$Na). Table 5 shows the heating values of different glycerol fractions and their corresponding viscosities at 40 °C. The HHV of glycerol increased from 17 MJ/kg for pure glycerol to 21 MJ/kg by adding 20% $C_{18:1}$Na, which is similar to the value for by-product glycerol from biodiesel production [24]. The HHV gradually increased toward 25 MJ/kg depending on the repeat count for the reuse of glycerol in delignification. The LHV increased from 20 MJ/kg for the unused 20% $C_{18:1}$Na glycerol solution to 23 MJ/kg for the used solution after a third delignification. It was assumed that these increases in heat values resulted from the accumulation of lignin in the glycerol.

The viscosities and viscosity indices of the glycerol fractions at 40 °C are also shown in Table 5. The viscosity of the glycerol fraction was 0.3 Pa s, but that of the 20% mixture of $C_{18:1}$Na in glycerol was not recorded because the mixture was a solid at 40 °C. After the first delignification, the viscosity of the glycerol solution was 7.6 Pa s, but this increased to 31.3 Pa s after the second round, and was then too high to record after the third round. These increases in viscosity were attributed to the dissolution of

Table 4 Condition of enzymatic saccharification of Japanese cedar and oak

Code	Symbols in Fig. 6	Substrate	Treatment conditions			Lignin content [wt%]
			Glycerol composition	Temp. [°C]	Time [h]	
Run 1	—✳—	Cellulose powder	–	–	–	0
Run 2	—○—	Japanese cedar	–	–	–	37.5
Run 3	—⬠—	Japanese cedar	20 wt% C_{12} Na	250	1	11.5
Run 4	—□—	Japanese cedar	20 wt% $C_{18:0}$ Na	250	1	15.5
Run 5	—◇—	Japanese cedar	20 wt% $C_{18:1}$ Na	250	1	9.3
Run 6	—△—	Japanese cedar	20 wt% C_2 Na	250	1	31.7
Run 7	—▽—	Japanese cedar	Only glycerol	250	1	35.2
Run 8	- -●- -	Oak	–	–	–	26.4
Run 9	- -◆- -	Oak	20 wt% $C_{18:1}$ Na	250	1	3.6
Run 10	- -▲- -	Oak	20 wt% C_2 Na	250	1	8.6

Table 5 Heating values and viscosities of glycerol fuels and effect of repeated delignification

Repeat count	Heating value		Viscosity	
	HHV [MJ/kg]	LHV [MJ/kg]	Absolute viscosity [Pa s]	Viscosity index [–]
Pure glycerol	17.8	15.8	0.3	0.99
20 wt % $C_{18:1}$ Na glycerol	21.7	20.0	ND	ND
1	20.5	19.2	7.6	0.73
2	23.1	21.2	31.3	0.66
3	25.0	23.3	ND	ND

Viscosity could not be measured because of solid state at 40 °C

ND not detected

lignin and hemicellulose in the $C_{18:1}$Na glycerol solution. The viscosity index for pure glycerol decreased from 0.99 to 0.73 and 0.66 for the $C_{18:1}$Na glycerol solutions after the first and second delignifications, respectively. This trend shows that the pseudoplastic nature of the fluid increased by the dissolution of lignin.

Conclusions

The delignification of wood powder using a mixture of glycerol and sodium soap was investigated as a pretreatment method for the production of bioethanol. The addition of 10–20% sodium soap to glycerol promoted the delignification of softwood, hardwood, and gramineous plants. The required temperature for delignification was dependent on the type of biomass. In particular, the delignification treatments of two softwood species (Japanese cedar and spruce) were successful at treatment temperatures above 250 °C under atmospheric pressure. The process of delignification with the glycerol–sodium soap mixture also promoted the enzymatic saccharification of the cellulosic residue. The glycerol fraction showed increased HHVs and LHVs after delignification owing to increased lignin content. Taken together, the results of this study suggest that glycerol–soap mixtures from biodiesel production can be successfully used in the delignification of hardwood and softwood.

Abbreviations

HHV: Higher heating value; LHV: Lower heating value; HPLC: High-performance liquid chromatography; XRD: X-Ray diffraction; C_2Na: Sodium acetate; C_{12}Na: Sodium laurate; $C_{18:0}$Na: Sodium stearate; $C_{18:1}$Na: Sodium oleate; $C_{18:2}$Na: Sodium linoleate; $C_{18:1}$: Oleic acid.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP19K15887 and 2018 grant from the Japan Soap and Detergent Association.

We thank Fuchigami Co., Ltd. for technical support regarding the calorific value measurements. We also thank Mr. Kiyokazu Inoue, Mr. Mao Watanabe, Mr. Ryota Sadakane, and Mr. Ryuya Kaji (undergraduate students at Fukuoka University) for assistance in completing this work. We are grateful to Assoc. Prof. Siaw Onwana-Agyeman of Tokyo University of Agriculture and Technology for checking a draft version of the manuscript.

Part of this research was presented at three conferences: the 68th Annual Meeting of the Japan Wood Research Society, Kyoto, Japan, March 14–16, 2018; the 2nd International Conference on Bioresource Technology for Bioenergy, Bioproducts, and Environmental Sustainability, Sitges, Spain, September 16–19, 2018; and the 25th Meeting of the Kyushu Branch of the Japan Wood Research Society, Fukuoka, Japan, November 15–16, 2018.

Authors' contributions

MT and MS generated the study design. MT mainly performed experiments and analysis. WK and HM discussed analysis data and the manuscript content. MS is the corresponding author. All authors read and approved the final manuscript.

Funding

This work was supported by JSPS KAKENHI Grant Number JP19K15887 and 2018 grant from the Japan Soap and Detergent Association.

Availability of data and materials

All data generated and analyzed are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Received: 11 June 2019 Accepted: 26 September 2019

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