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ORIGINAL ARTICLE

Concentrated sulfuric acid hydrolysis of softwood with *t*-butyl alcohol

Yuya Shiraki[1] · **Tomohiro Goto**[2] · **Hiroshi Nonaka**[1]

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Abstract
To date, concentrated sulfuric acid hydrolysis is the most effective process to recover the maximum yield of monomeric sugars from woody biomass because concentrated sulfuric acid can completely swell and hydrolyze cellulose. During this process, sulfuric acid lignin is quantitatively produced as a by-product, which is difficult to use because of self-condensation between the lignin molecules under acidic conditions. In this study, to achieve a high yield of both sugars and lignin, we aimed to valorize the lignin with a unique additive, *t*-butyl alcohol, in the concentrated sulfuric acid hydrolysis of softwood. When *t*-butyl alcohol was added, sugar yields were unchanged and were nearly equal to the amount of compositional sugars. Thermoplastic lignin with good solubility in acetone was successfully recovered in a yield higher than 40%. The economic efficiency of the concentrated acid hydrolysis was significantly improved by upgrading the lignin. Thus, this unique process could be a new candidate in the realization of sugar platform biorefineries.

Keywords Biorefinery · *t*-Butyl alcohol · Concentrated sulfuric acid hydrolysis · Lignin valorization · Self-condensation

1 Introduction

The saccharification of wood with concentrated sulfuric acid has been extensively studied to obtain monosaccharides that can be converted into bioethanol and other chemicals by fermentation. There has been considerable research of methods to produce sugars from biomass, such as dilute acid hydrolysis and pretreatment (including dilute acid, steam explosion, hydrothermal treatment, alkaline cooking, and ball milling), followed by enzymatic hydrolysis. Among them, saccharification with concentrated sulfuric acid has important advantages: low cost, low temperature, high sugar yields, and fast reaction [1–4]. A method for the recovery of waste sulfuric acid with ion-exchange resin films has also been studied for recycling [5–7]. On the other hand, the lignin that is obtained as a by-product, which is called sulfuric acid lignin (SAL), has no use. SAL is produced with high yield and high purity and is generally used to determine the lignin content for woody biomass [8, 9]. In this concentrated sulfuric acid saccharification, a carbocation occurs at the C_α position of the lignin, and the lignin molecules self-condense between the C_α and C_6 positions [10–12]. The lignin then loses its thermoplasticity and solubility in organic solvents; it becomes very difficult to utilize this lignin as a material. For the practical application of the concentrated sulfuric acid saccharification, it is imperative to reduce production costs, which can be realized by the use of the high-value-added lignin.

Funaoka et al. developed a functional lignin (lignophenol) that did not suffer from self-condensation by the concentrated sulfuric acid treatment with phenolic compounds (phase-separation system) [13, 14]. However, so far, alcohols have not been used with concentrated sulfuric acid although a variety of alcohols are applied with acid in organosolv processes to extract lignin. Moreover, alcohols are typically less toxic than phenolic compounds. This study investigates the applicability of *t*-butyl alcohol (TBA) as an additive for the concentrated sulfuric acid treatment. We also report a significant increase in SAL yield and SAL valorization and almost no reduction in sugar recovery.

✉ Hiroshi Nonaka
nonaka@bio.mie-u.ac.jp

[1] Graduate School of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie, 514-8507, Japan

[2] Faculty of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie, 514-8507, Japan

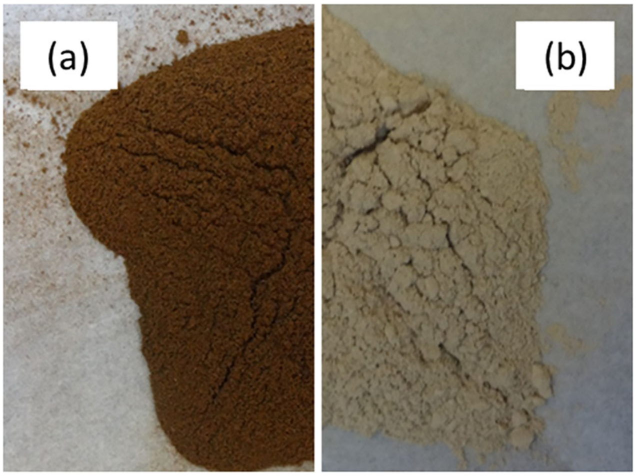

Fig. 1 Difference in lignin color between **a** SAL and **b** TBA-treated SAL

2 Materials and methods

2.1 Concentrated sulfuric acid hydrolysis of wood meal with TBA

Softwood wood meal (Hinoki cypress, 40-mesh pass) was extracted with ethanol/benzene (1:2, v/v) for 48 h. The water content of the extractive-free wood meal was 8.2% after oven-drying. A total of 8.27 g of TBA (Kanto Chemical Co., Inc., Tokyo), 0.86 g of deionized water, and 0.8 g of extractive-free wood meal were mixed, and 9 g of 95% sulfuric acid (Wako Pure Chemical Industries, Ltd., Osaka) was added little by little while the mixture was cooled. The reaction mixture was intensely stirred at 40 °C for 40 min. The reaction mixture was then diluted with deionized water to become equivalent to 4 wt% sulfuric acid, and it was boiled for 3 h. After boiling, 6 mL of 0.04 g/mL D-ribose (Wako Pure Chemical Industries, Ltd., Osaka) solution was added as an internal standard for sugar analysis.

The acid-insoluble fraction was recovered as TBA-treated SAL by the filtration of the hydrolysate with a glass filter (1G4), washed with deionized water until neutral, and dried overnight at 105 °C. As a reference, wood meal was simply treated with 11.88 g of 72% sulfuric acid, and the acid-insoluble fraction was similarly recovered as SAL.

Calcium carbonate was added to a portion of the filtrate until pH 6.8 was achieved to remove sulfuric acid. The supernatant was filtrated with a syringe filter (pore size 0.45 μm). Monosaccharides in the filtrates were quantified by Shimadzu's unique post-column fluorescence detection method by using a high-performance liquid chromatography (HPLC) instrument equipped with a Shim-pack ISA-07/S2504 reducing sugar analysis system (Shimadzu, Kyoto) [15].

2.2 Solubility of lignin in acetone

Acetone (3 mL) was added to 40-mg lignin sample, magnetically stirred for 120 min, and filtrated with a glass microfiber filter (Whatman GF/A, particle retention capacity 1.6 μm), and washed with 15 mL acetone. The filter was air-dried and oven-dried at 105 °C. The solubility of the lignin in acetone was calculated on the basis of the weight of the insoluble fraction.

2.3 Size exclusion chromatography of lignin

Lignin was dissolved in tetrahydrofuran (THF). Size exclusion chromatography (Shimadzu Class LC-20 system with a UV detector (280 nm), Shodex KF804, KF803, KF802, and KF801 in series) was performed using THF (with 15 mmol L^{-1} phosphoric acid) as the eluent at the flow rate of 1 mL/min. The polystyrene standard ($M_w = 400{,}000$; 200,000; 100,000; 50,000; 25,000; 7000; 3790; and 2000), bisphenol A ($M_w = 228$), and *p*-cresol ($M_w = 108$) were used to obtain the calibration curve.

2.4 Thermogravimetric analysis (TGA) of lignin

Lignin powder (~ 3 mg) was added to an aluminum pan, the surface of the powder was flattened, and TGA of the lignin was performed with a TG/DTA6200 instrument (Hitachi High-Technologies Co., Tokyo) from 100 to 500 °C (heating rate, 5 °C/min; nitrogen flow rate, 300 mL/min).

2.5 Thermomechanical analysis (TMA) of lignin

An Al plate with 1-mm diameter was placed on the surface of the smoothed lignin powder (~ 1 mm thickness) in an Al pan, and the penetration probe of a Thermo plus TMA8310 instrument (Rigaku Co., Tokyo) was set on the Al plate. TMA of the lignin was performed from 100 to 250 °C (heating rate, 5 °C/min; nitrogen flow rate, 200 mL/min; load, − 49.0 mN).

2.6 Fourier transform infrared spectroscopy (FT-IR)

After grinding and mixing lignin and potassium bromide (Nacalai Tesque, INC., Kyoto) in an agate mortar, a disk was prepared with a handy press (MHP-1, Shimadzu). The

Table 1 Yields of lignin and sugars (% of oven-dried extractive-free wood meal)

TBA	Lignin	Glucose	Mannose	Galactose	Xylose	Arabinose
Without	31.8 (0.3)	46.2 (1.3)	11.0 (0.8)	2.3 (1.2)	3.3 (0.3)	1.3 (0.6)
With	41.5 (0.8)	43.4 (1.1)	12.0 (0.3)	2.8 (1.1)	3.3 (0.4)	1.3 (0.5)

Values in parentheses "()" are standard deviation

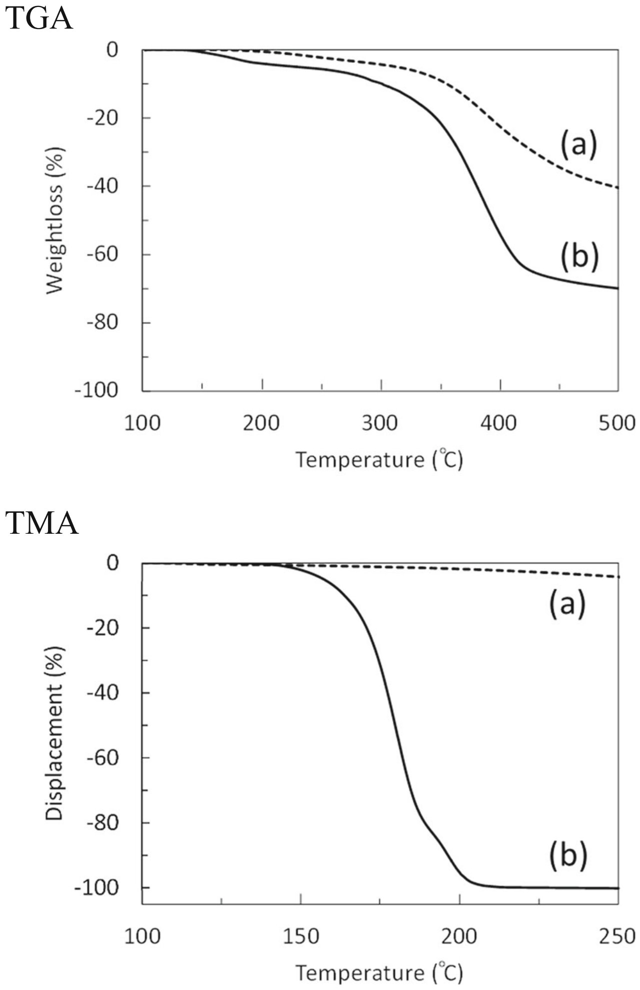

Fig. 2 TGA curves and TMA curves for **a** SAL and **b** TBA-treated SAL

FT-IR spectra were measured by using an FT-IR 8400S instrument (Shimadzu).

2.7 ^{1}H-NMR analysis

A total of 60 mg TBA-treated SAL was dissolved in 600 μL dimethyl sulfoxide-d$_6$ (DMSO-d$_6$) (FUJIFILM Wako Pure Chemical Corp., Osaka). ^{1}H-NMR analysis was performed for this solution using a JNM-ECZ500R (500 MHz) spectrometer (JEOL Ltd., Tokyo).

3 Results and discussion

Pictures of the recovered lignins are shown in Fig. 1. SAL is dark brown, whereas TBA-treated SAL is light brown. The yield of SAL was 31.8%, which is a typical value for the acid-insoluble lignin content of softwood. The yield of TBA-treated SAL was 41.5%, which is 9.7% higher than that of SAL (Table 1).

Although only 0.5% SAL was dissolved in acetone, more than 96% lignin dissolved due to TBA addition. These results showed that the self-condensation of lignin was prevented by TBA addition during the sulfuric acid treatment, which changed the solubility of SAL in organic solvents. More than 97% lignin dissolved in THF as well as acetone. The weight average molecular weight (M_w) and number average molecular weight (M_n) were calculated as 11,843 and 1887, respectively, relative to the polystyrene standards.

The TGA and TMA curves are shown in Fig. 2. In TMA of SAL, the curve was almost a straight line and glass transition was not observed. On the other hand, the TMA curve of lignin recovered using TBA shows displacement twice, at 168.4 °C and 193.8 °C. Because these temperatures are lower than the 5% weight decrease temperature in the TGA (229.6 °C), these are regarded as the glass transition temperature and melting temperature of the lignin, respectively. These results also support the conclusion that self-condensation of lignin was prevented by the addition of TBA during the sulfuric acid treatment. The SAL was successfully changed to thermoplastic lignin that is soluble in organic solvents. Lignin with thermoplasticity could be deployed in various applications, and we are particularly interested in applying this lignin to prepare lignin-based carbon molecular sieve membranes for hydrogen separation [16].

A significant increase in methyl groups at around 2950 cm^{-1} and tert-methyl groups at around 1370 cm^{-1} was clearly observed by the remarkable absorbance in FT-IR analysis (Fig. 3). which suggests that *t*-butyl groups were connected somewhere in the lignin during the treatment. Lignin did not lose

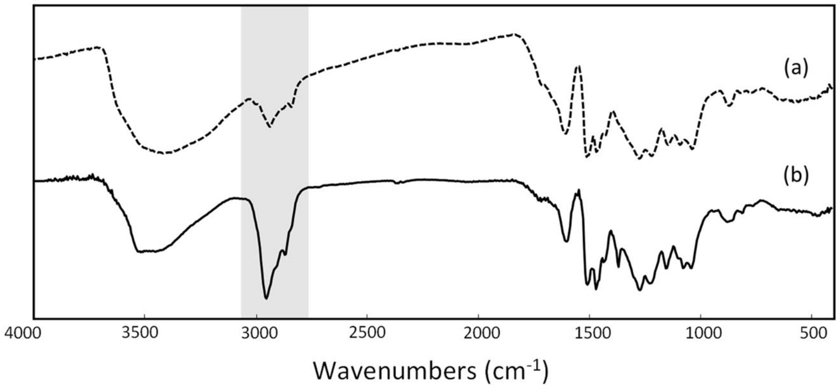

Fig. 3 FT-IR spectra for **a** SAL and **b** TBA-treated SAL

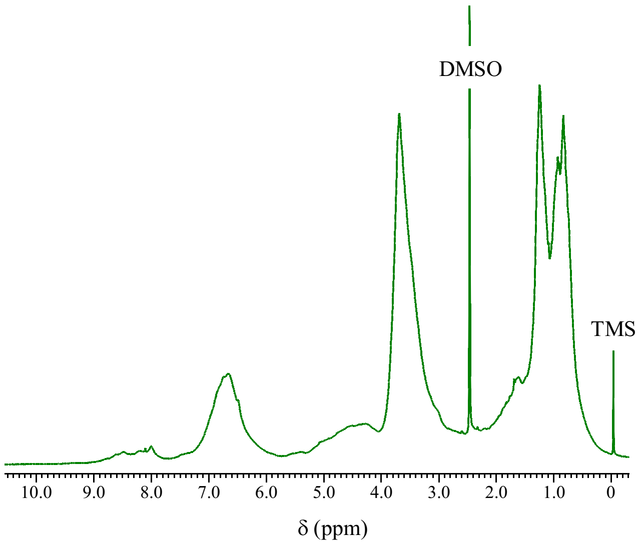

Fig. 4 ^{1}H-NMR spectrum for TBA-treated SAL

weight by further 72% sulfuric acid treatment, indicating that TBA is hardly ether-linked to lignin molecules. Because TBA is a tertiary alcohol, dehydration could more easily occur under acidic conditions, than other primary and secondary alcohols, to give *t*-butyl cations. In the beginning, we assumed that self-condensation between lignin molecules might be prevented by *t*-butylation at the C_6 position in lignin. However, it is impossible to attribute the ^{1}H-NMR signals from 0.8 to 1 ppm (Fig. 4) only by *t*-butyl groups. Under acidic conditions, it has been reported that *t*-butyl cations (Fig. 5, R^1 cation) are converted to isobutene, which reacts with *t*-butyl cations to form 1,1,3,3-tetramethylbutyl cations (Fig. 5, R^2 cation) [17, 18]. The ^{1}H-NMR spectrum indicates that the 1,1,3,3-tetramethylbutyl cations are the main reactants to prevent the self-condensation of lignin. Further analyses, such as using pyrolysis gas chromatography mass spectrometry and 2D-NMR, must be carried out to elucidate the reactions between lignin and TBA.

The yields of monosaccharides in the hydrolysate are compared in Table 1. Because 72% sulfuric acid hydrolysis is typically conducted for the determination of compositional sugars in biomass, the yields obtained without TBA addition almost correspond to the maximum values that we could achieve. On TBA addition, it seemed that the glucose yield slightly decreased and the yields of mannose and galactose might have slightly increased, indicating that the effect of the presence of TBA on the sugar yields is very small. The yields are maintained at high values, which is the most important advantage of concentrated sulfuric acid hydrolysis of wood. TBA may have interfered in the contact between sulfuric acid and polysaccharides, thereby slowing the hydrolysis rate to reduce overdecomposition of glucomannan as well as to slightly reduce glucose yield.

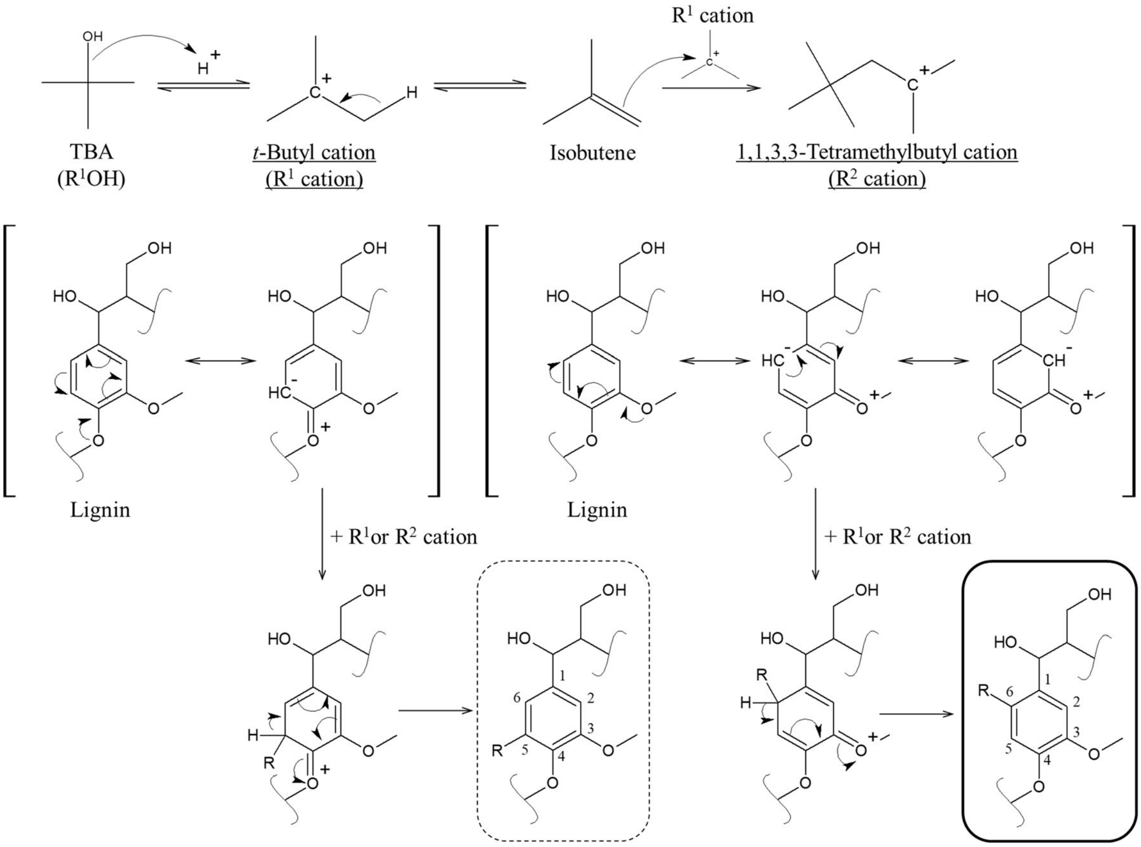

Fig. 5 Schematic representation of the estimated prevention of the self-condensation of lignin molecules in concentrated sulfuric acid by using *t*-butyl alcohol

TBA has low toxicity, and excess TBA can be removed by heating because of its low boiling point (~ 82 °C). The sugar solution could be utilized for fermentation after neutralization and removal of inhibiting compounds in a similar way as other biorefinery processes. In the future, the time for secondary hydrolysis with 4% sulfuric acid can be comprehensively optimized from the viewpoint of not only sugar yields but also fermentation inhibitors and residual TBA.

4 Conclusions

We succeeded in the high-yield production of sugars and thermoplastic lignin by the concentrated sulfuric acid treatment of softwood with TBA as an additive. A light brown lignin that is soluble in acetone was obtained in a yield higher than 40%. The sugar yields hardly changed after the treatment, which indicates that this process could produce sugars in a high yield, close to the theoretical maximum value. This study is the first to report the ability of TBA to upgrade lignin. Various reaction parameters, such as TBA amount, sulfuric acid concentration, and reaction time, will be further investigated to control the functionality of the lignin and maximize sugar yields. The economic efficiency of the concentrated acid hydrolysis process is considerably improved by upgrading the lignin. This unique process could be a new candidate in the realization of sugar platform biorefineries.

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