Catalytic Performance of Food Additives Alum, Flocculating Agent, Al(SO4)3, AlCl3, and Other Lewis Acids in Microwave Solvolysis of Hardwoods and Recalcitrant Softwood for Biorefinery

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ABSTRACT: The development of a novel pretreatment system using catalysts with high safety and low cost is pivotal to establish lignocellulosic biorefinery. We evaluated 16 Lewis acid catalysts for microwave solvolysis pretreatment to enhance enzymatic saccharification of woody biomass and found that very cheap and safe food additives, alum, are effective for the pretreatment, giving high sugar yield comparable to that of AlCl3, a strong Lewis acid catalyst effective both for softwood and hardwood. In microwave solvolysis of Japanese cedar, Paraserianthes falcataria and Eucalyptus globulus using alum in ethylene glycol/water (9/1, w/w), the maximum sugar yields after enzymatic saccharification reached 47.8, 51.0, and 59.7% based on the weight of each wood. The same reactions in glycol/water (9/1, w/w) gave the sugar yield, 34.5, 54.1, and 58.5%, indicating differential reactivity of the Lewis acid/solvent system depending on wood species. We found that efficiency and selectivity of pretreatment with a flocculating and astringent agent, Al(SO4)3, was promoted by microwave. Reaction of Japanese beech wood with Al2(SO4)3 in 50% aqueous 1-propanol by microwave and conventional heating revealed that microwave irradiation suppressed excessive degradation of carbohydrates into furfural derivatives. Saccharification of the wood pretreated by microwave with 8 and 1 filter paper units of cellulolytic enzymes gave sugar yields per wood of 50.3 and 43.9%, whereas the same reaction in an autoclave resulted in 48.9 and 34.7% yields, demonstrating that microwave irradiation accelerated the saccharification and the effect was remarkable with a lower enzyme dosage.

INTRODUCTION

The conversion of lignocellulosic biomass into biofuels, chemicals, energy, and new materials is becoming vital in solving the problems of greenhouse gas emissions and depletion of fossil fuels.1,2 In enzymatic saccharification and fermentation of lignocellulosic biomass, including woody biomass, disintegration of the plant cell wall is necessary to increase the accessibility of cellulolytic and hemicellulolytic enzymes to the cell wall polysaccharides, cellulose, and hemicelluloses. In this study, we developed new pretreatment reactions exhibiting microwave-sensitizer effects. Microwave pretreatment has drawn attention from the aspect of its rapid and direct heating, and reactions with acids, alkalis, and oxidative catalysts in aqueous and organic solvents have been applied for pretreatments of hardwood, softwood, and herbaceous biomass for enzymatic saccharification and fermentation.3−11

In microwave heating, conduction loss, dielectric loss, and magnetic loss are the major energy-transfer paths, but solution reactions with nonmagnetic molecules are mostly governed by dielectric loss and conduction loss heating. Both mechanisms require effective coupling between the components of the target material and the rapidly oscillating electric field of the microwaves. Dipole rotation is an interaction in which polar molecules align themselves with the rapidly changing electric field of the microwaves.12,13 Collisions and friction between the moving molecules result in heating. Heating by ionic conduction is similar to the dipole rotation path, except that these ions or ionic species interact with the oscillating electric field of the microwaves. The energy level of the microwave radiation is too low to produce direct chemical changes, such as breaking of covalent bonds and excitation of electrons. However, if the catalyst or substrates preferentially absorb the microwave energy, the energy absorbed is transformed from a radiation to chemical energy, forming high-temperature microscopic spots, which in many cases cause a large temperature gradient, thereby promoting chemical reactions that do not occur with conventional heating. Although the formation of hot spots in solution reactions is difficult to prove directly, acceleration of chemical reactions caused by microwave-absorbing spots on solid particles has been demonstrated.14−16 In Grignard reactions with electrically conductive Mg turnings, it was reported that an electric field significantly accelerates or deactivates the catalytic reaction depending on the applied electric field strength.17 Combination of CuO and H2O2 in alkaline media...
produced vanillin from softwood, and the reaction was accelerated by electric and magnetic fields with a slightly more prominent effect of electric fields. In some reactions, microwaves increase not only the reaction rate but also the selectivity of the reactions. Thus, microwave heating is useful if microwave-absorbing spots can be controlled by an electromagnetic field, and the reaction systems consist of catalysts, solvents, and substrates.

Lewis acids as classic catalysts are sometimes utilized for the pretreatment of biomass, The β-O-4 ether bond is the major mode of interunit linkages in lignin and a target for Lewis acid reactions. The catalyst coordinates with oxygen atoms of ethers to increase the dipole moment and to effectively cleave the ether bonds. In this study, we therefore focused on the potential of Lewis acids as microwave-sensitizer catalysts for the pretreatment of lignocellulosic biomass.

In the present study, we applied microwave-assisted pretreatments using Lewis acids to five species of wood, namely, Japanese cedar (Cryptomeria japonica), Japanese beech (Fagus crenata), Acacia mangium, Paraserianthes falcataria, and Eucalyptus globulus, and found significant pretreatment effects on all of the wood including the recalcitrant softwood, Japanese cedar. A comparative study of the Lewis acid reactions using microwave and conventional heating revealed that the disintegration of wood cell walls was promoted by microwave heating. The differential pretreatment effects on biomass conversion are reported.

**RESULTS AND DISCUSSION**

### Screening of Catalyst and Solvent

Fifteen Lewis acids were used to screen the catalysts for microwave-assisted pretreatment of woody biomass in 1-propanol/water (1/1, w/w). In addition to the Lewis acids, an organic carboxylic acid, maleic acid, was used to compare the efficiency with a Brønsted–Lowry acid with a low pKα. After pretreatment, soluble and insoluble (pulp) fractions were separated, and the pulp fraction was subjected to enzymatic saccharification. Sugar yields based on the weight of pulp and original wood are shown in Table 1. Aluminum Lewis acids effectively disintegrated the wood, giving pulp yields of 43.2–48.5% and high saccharification yields of 88.9–94.1% per pulp. Sugar yields based on the weight of original wood reached 40.0–43.2%, exceeding the yield using maleic acid. It is known that strong Lewis acids, including aluminum salts, are readily hydrated in a water-containing solvent system to form metal-hydroxide complexes such as [Al(OH)(H₂O)₃]²⁺ and [Al(H₂O)₄(OH)₂]⁻. The hydrated aluminum catalyzes a wide range of reactions such as reduction of ketones, reduction of carbon–carbon double bonds, oxidation of alcohols to carbonyls, and cleavage of ether and ester bonds. Cleavage of ether bonds probably plays a central role in the degradation of lignin.

Ferrie chloride gave high sugar yields comparable to those with Al₂(SO₄)₃. Zinc salts were less effective than the Al and Fe salts. The effects of the other eight metal salts were negligible. Among the series of metal chlorides examined, the decrease in pulp yield, an index of wood degradation, was in the order Al > Fe(III) > Zn(II) > Ca ≈ Na > Li. The order of counter anions in wood degradation was Br⁻ > Cl⁻ > SO₄⁻. These results are in accordance with the strength of the Lewis acids.

### Table 1. Comparison of 16 Pretreatment Chemicals Using Microwave Irradiation for Saccharification of Japanese Beech Wood

| catalyst       | pulp yield/wood % | saccharification ratio/pulp % | sugar yield/wood % |
|----------------|-------------------|-------------------------------|-------------------|
| (control)      | 84.2              | 16.7                          | 14.1              |
| LiCl           | 84.0              | 19.8                          | 16.6              |
| LiBr           | 83.5              | 20.1                          | 16.8              |
| NaCl           | 78.9              | 28.5                          | 22.5              |
| CaCl₂          | 78.2              | 32.7                          | 25.6              |
| AlCl₃          | 45.0              | 88.9                          | 40.0              |
| AlBr₃          | 43.2              | 94.1                          | 40.6              |
| Al₂(SO₄)₃      | 48.5              | 89.0                          | 43.2              |
| FeCl₃          | 49.0              | 88.1                          | 43.2              |
| ZnCl₂          | 77.7              | 41.8                          | 32.5              |
| ZnBr₂          | 66.2              | 57.4                          | 38.0              |
| ZnSO₄          | 75.4              | 41.9                          | 31.6              |
| MgSO₄          | 84.7              | 14.3                          | 12.1              |
| NH₄Cl          | 79.2              | 28.2                          | 22.4              |
| (NH₄)₂SO₄      | 82.3              | 20.7                          | 17.0              |
| NH₄Ac          | 83.7              | 10.3                          | 8.6               |
| maleic acid    | 66.0              | 58.8                          | 38.8              |

Sample conditions: [catalyst] = 60 μmol (as M⁺ or cation)/g wood; solvent, −propanol/water = 10 g/10 g; and treated wood, 1 g of beech wood powder. MW irradiation conditions: power, 400 W; temperature, 180 °C; and irradiation time, 30 min. No catalyst was added in the control experiment. All samples were saccharified without fibrillation after MW irradiation.

### Table 2. Comparison of Solvents in Pretreatment Using Microwave Irradiation for Saccharification of Japanese Beech Wood

| solvent                  | pulp yield/wood % | saccharification ratio/pulp % | sugar yield/wood % |
|--------------------------|-------------------|-------------------------------|-------------------|
| 2-propanol               | –                 | 67.4                          | 9.3               |
| 2-propanol               | +                 | 55.4                          | 81.9              |
| 1-octanol                | –                 | 76.5                          | 32.3              |
| 1-octanol                | +                 | 60.8                          | 72.4              |
| methyl ethyl ketone      | –                 | 92.4                          | 14.0              |
| methyl ethyl ketone      | +                 | 47.0                          | 94.6              |
| acetyl acetone           | –                 | 71.5                          | 48.0              |
| acetyl acetone           | +                 | 45.1                          | 94.4              |
| 1-butanol                | –                 | 83.7                          | 24.8              |
| 1-butanol                | +                 | 47.4                          | 91.5              |

Sample conditions: catalyst, Al₂(SO₄)₃ ([Al⁺³] = 0 (−) or 60 (+) μmol/g wood); solvent, organ./solvent/water (10 g/10 g); and treated wood, 1 g of Japanese beech wood powder. MW irradiation conditions: power, 400 W; temperature, 180 °C; and irradiation time, 30 min. All samples were saccharified without fibrillation after MW irradiation.
Acetyl acetone and 1-butanol gave similar sugar yields per pulp of over 90%, and a decreased sugar yield of 72.4% was obtained in the reactions with 1-octanol. However, because the solvent systems giving high saccharification yields per pulp gave lower pulp yields, significant differences in the overall sugar yield based on the weight of original wood were not found among the solvent systems used. Thus, we conclude that a wide range of organic solvents can be applied in the pretreatments with Lewis acids. Striking differences in the sugar yields were found between the reactions with and without catalysts in all of the solvent systems used.

**Differences between Microwave and Conventional Heating.** Beech wood powder was pretreated with Al$_2$(SO$_4$)$_3$ using microwave and autoclave heating and saccharified at 160 and 180 °C with 5 and 40 FPU of cellulase. As shown in Table 3, the microwave treatment gave higher sugar yields compared with conventional heating, especially at low reaction temperatures and/or low enzyme dosage. Microwave and autoclave heating with the same reaction temperatures, times, catalyst concentrations, and stirring speeds gave pulps with distinctive morphological differences. The microwave treatment gave a pulp sheet that was more finely delignified (data not shown).

We also compared the reactions of beech wood with Al$_2$(SO$_4$)$_3$ in 1-propanol/water (1/1, w/w) using microwave and conventional heating. The degradation products in the soluble part were analyzed by gas chromatography–mass spectrometry (GC–MS) (Figure 1). In the microwave reactions, six lignin-derived compounds, hydroxymethylfurfural (HMF), and monosaccharides were detected (Figure 1a). In the conventionally heated reactions, a variety of lignin degradation products with higher amounts of Hibbert ketones and coniferyl and sinapyl alcohols were found (Figure 1b). The amount of monosaccharide decreased as the HMF concentration increased, probably owing to the thermal degradation of carbohydrates. The amounts of benzyl alcohol, vanillin, and syringaldehyde were almost the same in the two reaction systems. Thus, clear differences in reactivity were found between microwave and conventional heating in biomass pretreatments using Lewis acids in aqueous organic solvents.

**Effects of Pretreatment Conditions on Sugar Yields from Enzymatic Saccharification of Softwood and Hardwood.** Five different wood species were pretreated by

![Figure 1. Comparison of degradation products by GC–MS analysis between microwave (a) and conventional heating (b) treatments. Sample conditions: catalyst, Al$_2$(SO$_4$)$_3$ ([Al$^{3+}$] = 60 μmol/g wood); solvent, 1-propanol/water (10 g/10 g); and treated wood, 1 g of Japanese beech wood powder; and treatment time, 30 min. All samples were saccharified without fibrillation after treatment.](image-url)
Figure 2. continued
Figure 2. continued
microwave solvolysis using AlCl₃ in ethylene glycol/water (9/1, w/w), and the effects of irradiation time, temperature, and catalyst concentration on enzymatic saccharification were analyzed. Figure 2A1–E1 demonstrates that the pulp yield decreased as the catalyst concentration and reaction temperature increased. We found that the sugar yield based on the weight of the original wood correlated well with the pulp yield, although the plots in Figure 2A2–E2 (40 FPU/g pulp) and 2A3–E3 (8 FPU/g pulp) originated from reactions with different amounts of catalyst and reaction temperatures. Enzymatic hydrolysis with a higher amount of cellulase allows high sugar yields with a wider range of pulp yields (Figure 2A2–E2), whereas the use of less enzyme considerably narrows the optimal range and results in a smaller range of pulp yields for all of the wood species (Figure 2A3–E3). In the treatment of Japanese cedar wood, the optimal range is much narrower than that for hardwood species. The maximum sugar yields of Japanese cedar, Japanese beech, A. mangium, P. falcataria, and E. globulus are 54.4, 54.7, 55.7, 63.1, and 60.8%, respectively, with 40 FPU/g pulp of cellulase and 48.3, 47.1, 52.7, 59.2, and 52.7%, respectively, with 8 FPU/g pulp of the enzyme.

**Pretreatment Using Lewis Acids with High Safety.**
Wood powders of Japanese cedar, P. falcataria, and E. globulus were pretreated with Al₂(SO₄)₃ and AlK(SO₄)₂ in ethylene glycol/water (Table 4) and glycerol/water (Table 5) (9/1, w/w each). The treatment of Japanese cedar using Al₂(SO₄)₃ and AlK(SO₄)₂ in aqueous ethylene glycol resulted in a high sugar yield, while the use of AlK(SO₄)₂ in glycerol/water produced lower sugar yields.
yield comparable to that with AlCl₃. These catalysts were reactive with *P. falcataria* and *E. globulus* wood, giving high sugar yields over 50 and 55%, respectively. When the same reactions were carried out in glycerol, the sugar yields decreased, except for the maximum sugar yields from *P. falcataria* and *E. globulus*. The catalyst Al₂(SO₄)₃ is used as a flocculating and astringent agent, and its LD₅₀ for oral administration in rats is >9000 mg/kg. AlK(SO₄)₂ is known as alum and is widely used as a food additive and in cosmetics owing to its high safety (LD₅₀: N/A). Glycerol (LD₅₀ = 12 600 mg/kg for oral administration in rats) is used for cosmetics and medical drugs. Thus, the combination of AlK(SO₄)₂ in glycerol/water is featured as the most eco-friendly and safe pretreatment system.

### CONCLUSIONS

Pretreatment of woody biomass by microwave solvolysis with aluminum salts, namely, AlCl₃, Al₂(SO₄)₃, and AlK(SO₄)₂ in aqueous organic solvents was developed. The reactions were applicable to both recalcitrant softwood and hardwood. In the reaction with AlCl₃, microwave irradiation accelerated the enzymatic saccharification, resulting in a higher amount of monosaccharides in the soluble fraction. Thus, microwave effects were found in the pretreatment system with the aluminum salts. The use of alum and aluminum sulfate in glycerol is featured as an eco-friendly reaction system using commodity chemicals with high safety and a low price.

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**Table 4. Treatment of Japanese Cedar, *P. falcataria*, and *E. globulus* with Al₂(SO₄)₃ and AlK(SO₄)₂ in an Ethylene Glycol/Water System**

| wood species | temperature/°C | catalyst | [Al³⁺]/μmol/g wood | pulp yield/wood % | saccharification ratio/pulp % | sugar yield/wood % |
|--------------|----------------|----------|---------------------|------------------|-------------------------------|-------------------|
| *Japanese cedar* | 180 | w/o | Al₂(SO₄)₃ | 0 | 100.1 | 0.0 | 0.0 |
| 60 | 92.6 | 0.1 | 0.1 |
| 120 | 58.2 | 64.4 | 37.5 |
| 180 | 49.5 | 94.4 | 46.7 |
| AlK(SO₄)₂ | 60 | 56.6 | 47.7 | 27.0 |
| 120 | 49.8 | 95.9 | 47.8 |
| 180 | 47.0 | 99.2 | 46.6 |
| 160 | w/o | 0 | Al₂(SO₄)₃ | 60 | 106.9 | 0.0 | 0.0 |
| 120 | 91.0 | 0.0 | 0.0 |
| 180 | 83.6 | 12.2 | 10.2 |
| AlK(SO₄)₂ | 60 | 93.0 | 0.0 | 0.0 |
| 120 | 78.3 | 16.2 | 12.7 |
| 180 | 78.9 | 19.7 | 15.5 |
| *P. falcataria* | 180 | w/o | Al₂(SO₄)₃ | 60 | 100.4 | 0.0 | 0.0 |
| 120 | 82.8 | 46.1 | 38.2 |
| 180 | 62.2 | 78.8 | 49.0 |
| AlK(SO₄)₂ | 60 | 62.4 | 79.6 | 49.6 |
| 120 | 52.2 | 97.6 | 51.0 |
| 180 | 48.7 | 99.3 | 48.4 |
| 160 | w/o | 0 | Al₂(SO₄)₃ | 60 | 102.8 | 0.2 | 0.2 |
| 120 | 88.9 | 32.3 | 28.7 |
| 180 | 74.6 | 63.8 | 47.6 |
| AlK(SO₄)₂ | 60 | 92.8 | 19.3 | 17.9 |
| 120 | 69.8 | 72.3 | 50.5 |
| 180 | 60.5 | 83.1 | 50.2 |
| *E. globulus* | 180 | w/o | Al₂(SO₄)₃ | 60 | 87.4 | 38.2 | 33.4 |
| 120 | 58.8 | 102.5 | 60.3 |
| 180 | 52.6 | 101.6 | 53.4 |
| AlK(SO₄)₂ | 60 | 52.3 | 100.3 | 51.0 |
| 120 | 46.5 | 109.0 | 50.7 |
| 180 | 48.7 | 110.0 | 53.6 |
| 160 | w/o | 0 | Al₂(SO₄)₃ | 60 | 95.3 | 0.2 | 0.2 |
| 120 | 92.6 | 28.2 | 26.1 |
| 180 | 68.3 | 84.9 | 57.9 |
| AlK(SO₄)₂ | 60 | 58.2 | 97.7 | 56.8 |
| 120 | 65.1 | 91.7 | 59.7 |
| 180 | 55.8 | 103.8 | 57.9 |
| 160 | w/o | 0 | Al₂(SO₄)₃ | 60 | 95.3 | 0.2 | 0.2 |
| 120 | 92.6 | 28.2 | 26.1 |
| 180 | 68.3 | 84.9 | 57.9 |

Sample conditions: solvent, ethylene glycol/water = 18 g/2 g and treated wood, 1 g of wood powder. MW irradiation conditions: power, 400 W; temperature, 160 and 180 °C; and irradiation time, 30 min. All samples were saccharified without fibrillation after MW irradiation.
MATERIALS AND METHODS

Wood Samples and Materials. The sap wood of five wood species, *C. japonica* (Japanese cedar), *F. crenata* (Japanese beech), *A. mangium*, *P. falcataria*, and *E. globulus*, was cut into chips. The chips were Wiley-milled, and the powder formed was sieved to collect 14−30 mesh-sized particles. The wood particles were air-dried to approximately 10% moisture content and used throughout this study.

Japanese cedar, Japanese beech, *A. mangium*, *P. falcataria*, and *E. globulus* contain 31.0, 23.0, 21.2, 26.5, and 22.2% of Klason lignin, 47.8, 49.9, 59.7, 52.5, and 46.6% of cellulose, and 20.3, 29.3, 20.1, 21.0, and 30.2% of hemicelluloses, respectively.7,8,32,33 Meicelase from *Trichoderma viride* (Meiji Seika Co., Ltd.) was used as a saccharification enzyme. The activity of the enzyme in filter paper units (FPU) was determined using an IUPAC method.34 All chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan).

Microwave Irradiation. Wood powder (1 g) in dry weight equivalent, a catalyst, and 20 g of mixed solvent were placed in a pressure-tight vial for microwave irradiation. After sealing the vial, it was microwave-irradiated using a Biotage Initiator 60 EXP instrument (Uppsala, Sweden) at 140−180 °C for 10−30 min with magnetic stirring at 900 rpm. The microwave power was automatically optimized by the heating machine within the range 0−400 W to adjust the temperature and irradiation time. The solution was vacuum-filtered with Advantec no. 131 filter paper (3 μm pass-through). The residual pulp was washed with...

Table 5. Treatment of Japanese Cedar, *P. falcataria*, and *E. globulus* with Al₂(SO₄)₃ and AlK(SO₄)₂ in a Glycerol/Water System

| wood species | temperature/°C | catalyst | [catalyst]/μmol/g wood | pulp yield/wood % | saccharification ratio/pulp % | sugar yield/wood % |
|--------------|----------------|----------|------------------------|------------------|-------------------------------|-------------------|
| Japanese cedar | 180 | w/o | 0 | 95.1 | 0.0 | 0.0 |
| | | | 60 | 89.0 | 0.3 | 0.3 |
| | | | 120 | 69.3 | 25.3 | 17.5 |
| | | | 180 | 61.4 | 40.1 | 24.7 |
| | | | 60 | 74.4 | 20.6 | 15.3 |
| | | | 120 | 61.4 | 49.4 | 30.3 |
| | | | 180 | 57.6 | 59.9 | 34.5 |
| | 160 | w/o | 0 | 108.9 | 0.0 | 0.0 |
| | | | 60 | 96.2 | 0.0 | 0.0 |
| | | | 120 | 94.1 | 0.1 | 0.1 |
| | | | 180 | 90.4 | 0.3 | 0.3 |
| | | | 60 | 84.2 | 0.0 | 0.0 |
| | | | 120 | 85.4 | 0.2 | 0.2 |
| | | | 180 | 85.9 | 0.5 | 0.4 |
| P. falcataria | 180 | w/o | 0 | 93.6 | 6.8 | 6.4 |
| | | | 60 | 83.7 | 45.2 | 37.8 |
| | | | 120 | 66.1 | 79.7 | 52.7 |
| | | | 180 | 54.8 | 95.9 | 52.6 |
| | | | 60 | 64.2 | 84.3 | 54.1 |
| | | | 120 | 55.8 | 96.6 | 53.9 |
| | | | 180 | 50.7 | 101.1 | 51.2 |
| | 160 | w/o | 0 | 104.7 | 0.0 | 0.0 |
| | | | 60 | 99.9 | 0.0 | 0.0 |
| | | | 120 | 97.0 | 8.8 | 8.6 |
| | | | 180 | 87.1 | 28.5 | 24.8 |
| E. globulus | 180 | w/o | 0 | 81.8 | 49.6 | 40.6 |
| | | | 60 | 58.7 | 92.4 | 54.2 |
| | | | 120 | 55.3 | 96.2 | 53.2 |
| | | | 180 | 53.8 | 95.5 | 51.4 |
| | | | 60 | 54.6 | 98.6 | 53.8 |
| | | | 120 | 49.5 | 103.4 | 51.2 |
| | | | 180 | 47.1 | 103.1 | 48.6 |
| | 160 | w/o | 0 | 97.2 | 0.0 | 0.0 |
| | | | 60 | 93.4 | 23.7 | 22.2 |
| | | | 120 | 74.7 | 67.7 | 50.6 |
| | | | 180 | 69.4 | 72.0 | 50.0 |

“Sample conditions: solvent, glycerol/water = 18 g/2 g and treated wood, 1 g of wood powder. MW irradiation conditions: power, 400 W; temperature, 160 and 180 °C; and irradiation time, 30 min. All samples were saccharified without fibrillation after MW irradiation.”
acetone and water, and the pulp yield was calculated using the following equation

\[
\text{(pulp yield)}/\% = \frac{(\text{treated pulp weight})/g}{(\text{original pulp weight})/g} \times 100
\]

**Enzymatic Saccharification and Determination of Reducing Sugar Concentration.** The pulp fraction (0.2 g, in dry weight equivalent), 50 mM acetate buffer (pH 4.5, 10 mL), and Me celase were placed in a 50 mL centrifuge tube and incubated at 45 °C, 140 rpm for 48 h using a reciprocal-shaking thermostatic bath. The amount of enzyme is described in each experimental section and the figure legends. After the reaction, the reducing sugar concentration was determined by the Somogyi–Nelson method.\(^{35}\) Sugar yields based on the weight of pulp and original wood were calculated using the following equations

\[
\text{(saccharification ratio)}/\% = \frac{(\text{obtained sugar weight})/g}{0.2 \text{ g}} \times 100
\]

\[
\text{(sugar yield)}/\% = \left(\frac{\text{pulp yield}}{}\right) \times \left(\frac{\text{saccharification ratio}}{100}\right)
\]

**Analysis of Lignin Degradation Products.** Lignin degradation products in the filtrate were determined by GC–MS after acetylation, trimethylsilylation, and/or O-(2,3,4,5,6-pentafluorobenzyl)oxime (PFBO) derivatization. One millimole of fluoranthene (100 μL in dichloromethane) was added to the filtrate (10–40 μL, depending on the concentration of lignin fragments) in a small vial, and the mixture was evaporated in vacuo before derivatization. In acetylation, acetic anhydride and pyridine (200 μL each) were placed in the vial and reacted at 60 °C for 1 h. In trimethylsilylation, N,O-bis(trimethylsilyl) trifluoroacetamide (100 μL) and pyridine (200 μL) were placed in the vial and reacted at 60 °C for 1 h. In PFBO derivatization, 5 mg/mL of O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA, 100 μL in methanol) was placed in the vial and reacted at room temperature overnight. After derivatization, the solution was evaporated and ethyl acetate (100 μL) was added. The solution was then filtered using GL Chromatodisk 4P (0.45 μm pass-through) to remove precipitates. The filtrate (1 μL) was subjected to GC–MS analysis. The analysis was carried out with a Shimadzu GCMS-QP5050A GC–MS (Kyoto, Japan) on a DB-1 column (length, 30 m; i.d., 0.25 mm; thickness, 1 μm; J&W Scientific Inc., CA). Electron impact mass spectrometry was performed at an ionization energy of 70 eV. The column oven temperature was raised from 90 to 240 °C at 10 °C/min, then from 240 to 310 °C at 30 °C/min, and then maintained for 10 min.

**Screenings of Catalyst and Solvent for Microwave Treatment.** To evaluate the efficiency of the catalysts, LiCl, LiBr, NaCl, CaCl₂, AlCl₃, AlBr₃, Al₂(SO₄)₃, FeCl₃, ZnCl₂, ZnBr₂, ZnSO₄, MgSO₄, (NH₄)₂SO₄, NH₄Ac, and maleic acid were used at a concentration of 60 μmol/g of wood for the pretreatment of beech wood powder in 1-propanol/water (1/1, w/w). In reactions with Al₂(SO₄)₃, five solvent systems, 2-propanol/water, 1-octanol/water, methyl ethyl ketone/water, acetyl acetone/water, and 1-butanol/water (1/1, w/w each) were used. Reactions without catalysts were also conducted as control experiments.

**Comparative Experiments with Microwave and Conventional Heating.** Beech wood powder was pretreated by microwave and conventional heating at 160 or 180 °C for 30 min using Al₂(SO₄)₃ (60 μmol/g of wood) in 1-propanol/water (1/1, w/w), and the components in aqueous organic solution and the sugar yields after enzymatic saccharification with two different amounts of cellulase (5 or 40 FPU/g of pulp) were compared. In the two reaction systems, the diameter of the reaction vessel and the stirring rate were the same.

**Optimization of Pretreatment Conditions in Microwave Solvolysis with AlCl₃.** The effects of the microwave irradiation conditions and the concentration of the Lewis acid catalyst were analyzed to determine the maximum sugar yields from the five wood species. Microwave irradiation was carried out at 140–180 °C for 10–30 min using AlCl₃ (60–720 μmol/g of wood) in ethylene glycol/water (9/1, w/w). The pulp fraction was subjected to enzymatic saccharification with 5 or 40 FPU of cellulase per gram of pulp.

**Microwave Pretreatment Using Lewis Acid Catalysts with Higher Safety.** Wood powders of Japanese cedar, P. falcata, and E. globulus were pretreated using AlK(SO₄)₂ or Al₂(SO₄)₃ (60–180 μmol/g of wood) in ethylene glycol/water or glycerol/water (9/1, w/w). Microwave irradiation was carried out at 160 and 180 °C for 30 min. The amount of saccharification enzyme is 40 FPU/g of pulp.

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**NOTES**

1. *Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan.

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