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Dissolution of wood in α-keto acid and aldehydic carboxylic acids and fractionation at room temperature

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Wood pulverised using a ball mill was dissolved in α-keto acid, pyruvic acid, and two aldehydic carboxylic acids, namely, glyoxylic acid and formic acid, at room temperature. Viscosity and optical transmittance analysis revealed that the wood particles dissolved in glyoxylic acid and formic acid in several hours, while they dissolved in pyruvic acid in several days. Size exclusion chromatography demonstrated that substances with number average molecular mass exceeding 10^5 were present in every solution. Incubation of these carboxylic acids with methanol produced the corresponding ester and/or (hemi)acetal derivatives, suggesting that the dissolution mechanism involves the substitution of hydroxyl groups in biomass. After dissolution, all solutions demonstrated non-Newtonian fluid behaviour with a non-Newtonian index n ranging from 0.41 to 0.72. When the dissolution time increased, the non-Newtonian index of the formic acid solution increased towards the value for a Newtonian fluid ($n = 1$). This rise was accompanied by partial depolymerisation. Evaporation of the wood solution gave a smooth transparent film. Using the water-immiscible solvent 2-methyltetrahydrofuran (2-MeTHF), the formic acid solution was separated into cellulosic porous solids, lignin-rich 2-MeTHF-soluble fraction and other components at room temperature. The dissolution of woody biomass at room temperature creates a new biorefinery process for the conversion of wood components in various solution reactions. In particular, in this study, pyruvic acid, which plays a central role in the metabolism of living organisms, is used as a new solvent for wood; thus, bridging biochemistry and wood biorefinery.

Introduction

Widely available in large quantity and non-competing with food supply, woody biomass is expected to serve as a platform for green chemicals and effectively mitigate greenhouse gas emission effects. The separation and conversion of its cell wall components have emerged as important technologies for the sustainable production of renewable fuels and chemicals. Major components of woody biomass comprise cellulose, hemicelluloses and lignin, which is a heterogeneous aromatic polymer. Cell wall polysaccharides are coated with lignin; thus, their dissolution and separation is difficult and hinders their utilisation. In 2003, ball-milled wood was dissolved in dimethyl sulphoxide (DMSO) mixtures with *N*-methylimidazole or tetrabutylammonium fluoride, and the components were analysed by NMR spectroscopy.[1] This pioneering report stimulated numerous studies addressing the solubilisation of ball-milled wood in various solvent systems such as DMSO/LiCl,[2] DMSO/ pyridine,[3] urea and NaOH/boric acid.[4] The effectiveness of ionic liquids has also been examined,[5] but solvent cost limits their industrial implementation.

Simple, biomass-derived and highly biodegradable chemicals that dissolve and separate woody biomass under mild conditions are attractive for the creation of a new biorefinery

process. This study aims at harnessing these solvent properties to develop a new wood solubilisation and fractionation technology at room temperature. Here, we report the dissolution of ball-milled wood in highly biodegradable acids such as pyruvic, glyoxylic and formic acids at room temperature; moreover, the resulting solution in formic acid was fractionated by precipitation. These solvents are biologically important compounds produced by various enzymatic reactions as well as organic synthesis. The simplest α-keto acid, pyruvic acid, is a biologically and chemically synthesised compound that is important for the metabolism of glucose in living organisms. The simplest carboxylic acid, formic acid, has been produced by free radical reaction of oxalic acid and thermochemical treatment of plant biomass. Glyoxylic acid, which is an intermediate of the glyoxylate cycle, has also been synthesised by oxidation of glyoxal. In aqueous solutions, glyoxylic acid has an equilibrium with its hydrated form.

Formic acid has been used to delignify corn cob at 60 °C[6] and birch wood at boiling temperature.[7] Lignin has been extracted from Miscanthus[8] and sugarcane bagasse[9] with refluxing formic acid solutions containing hydrochloric or sulphuric acids. The hydrolysis of pulp was also conducted in formic acid between 180 and 220 °C.[10] Glyoxylic acid has dissolved cellulose above 50 °C;[11] however, no report has addressed the dissolution of

wood by these carboxylic acids without additives at room temperature. Pyruvic acid is a new solvent for woody biomass that connects biological metabolisms and wood biorefinery. This study provides a new process that dissolves whole wood at room temperature and separates porous cellulosic solids from the solution by precipitation using water and 2-methyltetrahydrofuran (2-MeTHF), a water-immiscible solvent derived from furfural produced from biomass. Precipitation with 2-MeTHF facilitates the separation between solid, water and solvent; thus, enabling solvent recovery with minimum energy input. Therefore, this new solubilisation and fractionation process is environmentally benign.

Experimental

Materials and general methods

Chemicals were purchased from Wako Pure Chemical Industry Co., Ltd. (Osaka, Japan), Nacalai Tesque Inc. (Kyoto, Japan) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).
Eucalyptus globulus wood chips were purchased from Oji paper Co., Ltd. (Tokyo, Japan). The wood chips were ground to 20 mesh using a Wiley mill, extracted with ethanol/toluene (1:2, v/v) for 10 h and dried at 105 °C overnight. The dried wood particles were ground using a ball mill for 48 h under nitrogen atmosphere.
The water content of the ball-milled wood was determined to be 7.85% ± 0.05% after weight reduction by drying at 105 °C for 19 h. Yields were calculated based on the initial amount of milled wood excluding the water content. The average and standard deviation of three experimental data were determined for non-filterable material quantification, viscosity, and optical transmittance measurements.

Dissolution or dispersion of milled wood in carboxylic acids by immersion

Dissolution in liquid carboxylic acids was achieved by adding milled wood (0.2 g) to the carboxylic acid (4 g) with stirring at room temperature. For solid carboxylic acids, the acids were diluted with distilled water to prepare saturated aqueous solutions and milled wood (0.2 g) was dissolved in the resulting aqueous carboxylic acid solution (4 g). In each case, a 4.8% (w/w) solution was obtained after complete dissolution. The volume of the mixture was scaled up for viscosity measurements. For UV-visible spectroscopic measurements, the 4.8% solutions were diluted 100 or 200 times with the acid used for dissolution.

Reaction between acids and methanol

Pyruvic acid (0.44 g, 5.0 mmol) and methanol (0.24 g, 7.5 mmol) were mixed at room temperature for 30 min. Next, a small portion of the mixture (5–10 mg) was dissolved into CDCl$_3$ (0.5 mL) and transferred into a 5-mm NMR tube for analysis. Similarly, mixtures of glyoxylic acid monohydrate (0.23 g, 2.5 mmol) and methanol (0.12 g, 3.75 mmol) as well as formic acid (0.46 g, 10 mmol) and methanol (0.48 g, 15 mmol) were prepared and analysed by NMR spectroscopy.
The ^{1}H NMR spectra were recorded on a JNM Lambda-400 spectrometer (JEOL Ltd., Tokyo, Japan) at 400 MHz using tetramethylsilane as an internal reference. The products (**1**–**5**) were quantified by ^{1}H NMR spectra. Yields were calculated using integrals of the ^{1}H NMR peaks corresponding to the remaining unreacted acid and the newly formed products. In

reactions involving glyoxylic acid, yields were calculated from the amount of unreacted methanol because the amount of unreacted acid could not be directly determined by NMR spectroscopy.
Gas chromatography mass spectrometry (GC–MS) analysis was performed using Shimadzu GCMS-QP5050A system (Shimadzu, Co., Ltd., Kyoto, Japan) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific). The GC oven temperature was maintained at 50 °C for 3 min, then increased to 300 °C at heating rate of 8 °C min^{-1} and maintained at 300 °C for 20 min. Helium was used as carrier gas at a flow rate of 1.6 mL min^{-1}. The mass spectrometer was operated in the electron impact ionization mode at 70 eV for a mass/charge (*m/z*) scan range of 40–600. The split ratio was 1:10, and the interface temperature was set at 300 °C.
The products were identified by comparing the NMR and mass peaks with data reported in the Wiley and SDBS libraries (http://sdbs.db.aist.go.jp (National Institute of Advanced Industrial Science and Technology), 2013/10).

Quantification of soluble and non-filterable materials

Milled wood and formic acid were mixed and stirred for 1, 18 or 24 h. The mixture was then filtered under reduced pressure through a glass filter (ADVANTEC, GA-100, Φ 5.5 cm), which was pre-dried for 1 h at 105 °C. The collected non-filterable materials were dried overnight with a vacuum freeze dryer and weighed. The filtrate was concentrated to dryness using a rotary evaporator and weighed.

Viscosity and fluid property measurements

Viscosity of the solubilised samples was determined using a TVE-25H rotating viscometer (Toki Sangyo Co, LTD, Tokyo, Japan) equipped with a standard cone plate rotor (1°34′ × R24) in the mode H (η < 1,296 mPa s) or mode U ($\eta \geq$ 2,592 mPa s). Measurements were performed at a rotational speed of 5 rpm for 1 min. The viscometer temperature was maintained at 30 °C by water circulation using a MC-01 micro chiller (Advanced thermal sciences, CA, USA).
For fluid property investigations, viscosities were measured at different rotational speeds (0.5, 1, 2.5, 5, 10 and 20 rpm) in the mode U under the same conditions, unless the viscosity became out of range at high rotational speeds.
The shear rate $\dot{\gamma}$ (s^{-1}) was calculated by multiplying the value of rotational speed (rpm) by the shear rate coefficient 3.83 (for a standard rotor). Shearing stress σ (Pa) was obtained by multiplying the machine reading (%) by the shearing stress coefficient 1.986 (mode U). Thus, the viscosity η (Pa s) was then determined using equation (1):

$$\eta = \sigma/\dot{\gamma}. \quad (1)$$

According to the Ostwald–de Waele relationship,[12,13] the shear rate $\dot{\gamma}$ can be related to σ or η, as shown in equations 2 and 3:

$$\sigma = K\dot{\gamma}^{\,n}, \quad (2)$$
$$\eta = K\dot{\gamma}^{\,n-1}, \quad (3)$$

where K is the consistency index and n is the non-Newtonian (or flow behaviour) index calculated by the power approximation.

Optical transmittance measurements

About 4 g of liquid was placed in a test tube (inner diameter: 16 mm) and the transmittance T (%) was measured at 660 nm using a Miniphoto 518R optical density (OD) monitor

(TAITEC, Saitama, Japan). The transmittance of an opaque liquid showing a negative reading was assumed to be 0.

Determination of molecular weight distributions by size exclusion chromatography (SEC)

A mixture of milled wood and acid was diluted with dimethylformamide (DMF, 80 mg/mL) and the solution was filtered through a 0.45 μm polytetrafluoroethylene filter. Average number molecular mass (Mn) distributions were determined using a Prominence SEC system, (Shimadzu corporation, Kyoto, Japan) equipped with a photodiode array detector SPD-M20A set at 280 nm, a refractive index detector RID-10A and two Shodex KD-803 columns (8.0 × 300 mm, Showa Denko KK, Tokyo, Japan) linked to a guard column. A 10 μL sample was injected and analysed at 40 °C (column temperature) using DMF as an eluent at a flow rate of 0.8 mL min^{-1}. The columns were calibrated using polyethylene glycol and polyethylene oxide standards presenting molecular masses ranging from 586 to 107,000 g mol^{-1}. For the calibration, the time difference between UV and RI detectors (0.2 min) was corrected using a polystyrene standard.

UV-visible light absorption measurements

UV-visible absorption spectra of the milled wood solution were recorded between 190 and 800 nm on a U-3310 spectrophotometer (HITACHI, Ltd., Tokyo, Japan) at a scan speed of 300 nm/min in wavelength scan mode using a quartz cell (light path length: 10.0 mm).

Fractionation

Wood components in a ball-milled wood solution in formic acid were separated using a water-immiscible solvent, 2-MeTHF. Water and 2-MeTHF were added to the milled wood solution and fractionation was performed as shown in Figure 1. The water and 2-MeTHF solutions phase-separated and precipitates were formed. The upper 2-MeTHF layer was isolated and the lower aqueous suspension was filtered to separate the water layer from the precipitate. The precipitate was freeze-dried to obtain fraction 1 while water and 2-MeTHF layer were evaporated and freeze-dried to obtain fractions 2 and 3, respectively. Phase separation profiles of ball-milled wood solutions in glyoxilic and pyruvic acids were also examined by addition of water and 2-MeTHF at various weight compositions

Pyrolysis GC–MS of ball-milled wood fractions obtained from the formic acid solution

Compounds in each fraction were analysed by pyrolysis gas chromatography mass spectrometry (Py–GC–MS). The pyrolysis was performed using a pyrolyser PY-2020D (Frontier Laboratories, Fukushima, Japan) connected to a Shimadzu GC–MS QP5050A system (Shimadzu, Co., Ltd., Kyoto, Japan) equipped with a DB-5MS column (30 m × 0.25 mm i.d., film thickness: 0.25 μm; J&W Scientific). The sample (*ca.* 0.1 mg) was placed in a small cup, which was placed in a pyrolysis chamber. The pyrolysis was performed at 500 °C for 12 s and the interface temperature was 250 °C. Subsequently, the products were introduced into the GC–MS system with a split ratio of 1:97. The GC–MS analysis was performed using helium (1.5 mL min^{-1}) as carrier gas. The GC oven temperature was maintained at 45 °C for 4 min, increased to 300 °C at a heating rate of 4 °C min^{-1} and maintained at 300 °C for 20 min. The mass spectrometer was operated in the electron impact

ionization mode at 70 eV for *m*/*z* scan range of 40–600. Products were identified by comparing their mass peaks with spectral data from the Wiley7 and NIST107 libraries, and other reports.[14–17]
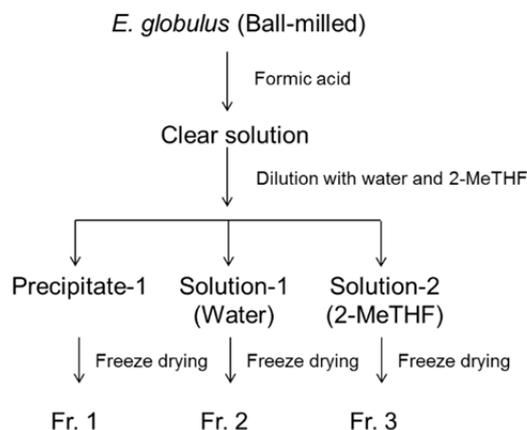
Fig. 1 Fractionation of a ball-milled wood solution in formic acid diluted with water and 2-MeTHF.

Compositional analysis of the fractions

The holocellulose content was determined according to the Wise chlorite method.[18] Klason lignin was determined from the weight ratio of solid residue after a two-step acid hydrolysis using 72% and 1.9% sulphuric acid according to TAPPI T222 om-98 procedure. The acid-soluble lignin in the filtrate was determined at 204 nm using a UV-vis spectrophotometer U-3310 (HITACHI, Ltd., Tokyo, Japan) after 10-fold dilution using 3% H_2SO_4 according to Tappi UM 250. An absorption coefficient of 106 Lg^{-1}cm^{-1} was used.[19]

Mono sugars obtained by neutralization following the two-step acid hydrolysis were characterised by HPLC equipped with a post-column and a fluorometric detector[20] using the Prominence HPLC system (Shimadzu, Co., Ltd., Kyoto, Japan) equipped with two Aminex HPX-87P columns (Bio-Rad, CA, USA) connected to two guard columns. For fluorescence detection, the samples were labelled at 150 °C using an aqueous solution of L-arginine (1%) and boric acid (3%). The sample solution (5 μL) was injected and analysed at 58 °C using water as an eluent at a flow rate of 0.3 mL min^{-1}.

Scanning electron microscopy

Scanning electron microscopy (SEM) images of the original ball-milled *E. globulus* and the glass filter before and after filtration of the formic acid solution were taken using a 3D Real Surface View Microscope VE-9800 (Keyence Corporation, Osaka, Japan). The sample was mounted on a sample stub using a double-sided carbon tape and gold-coated using a magnetron sputter coater MSP-1S (Vacuum device Inc., Ibaraki, Japan). SEM image of the precipitate (Fr. 1) obtained by 2-MeTHF/water fractionation of the wood solution in formic acid was taken.

Results and discussion

Dissolution of ball-milled wood in carboxylic acids

The solubility of the ball-milled wood in various carboxylic acids was investigated by immersion in the acid solutions with stirring at room temperature. The ball-milled wood showed high solubility in 64% aqueous glyoxylic acid solution (glyoxylic acid monohydrate: water = 80:20) as well as pyruvic and formic acids (Figure 2). However, DL-lactic acid, acetic acid and aqueous oxalic acid did not dissolve the milled wood.
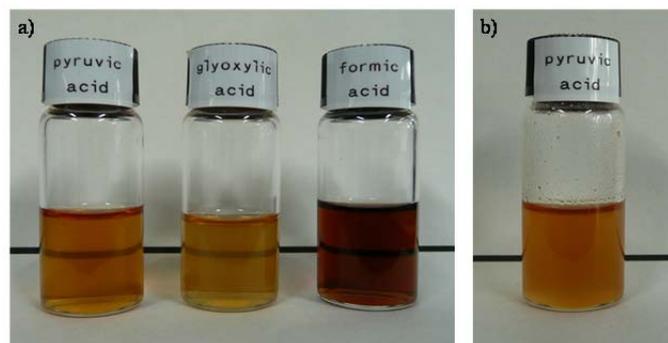
Fig. 2 Photographs showing ball-milled wood mixed with pyruvic acid, aqueous glyoxylic acid and formic acid at a concentration of 0.5%. a) Clear solution obtained using pyruvic, aqueous glyoxylic and formic acids 1 week after mixing. b) Suspension obtained 1 day after mixing with pyruvic acid.

Table 1 Solubility of the ball-milled wood in various carboxylic acids after standing for 7 days[a]

	pKa[21]	Acid concentration (w/w %)	Optical transmittance T(%), 660 nm	Solubility
Oxalic acid/water	1.25, 3.81	9.0	0.05 ± 0.03	Insoluble
Pyruvic acid	2.39	100	7.99 ± 0.5	Soluble
Glyoxylic acid/water	3.18	64	7.28 ± 1.9	Soluble
Formic acid	3.75	100	1.65 ± 0.2	Soluble
DL-lactic acid	3.86	100	0.03 ± 0.03	Insoluble
Acetic acid	4.76	100	0.006 ± 0.00	Insoluble

[a] The solubility was evaluated by optical transmittance

The dissolution of the milled wood was evaluated by measuring the transmittance of the mixture after 7 days (Table 1). The milled wood dissolved readily in pure pyruvic and formic acids but only partially in aqueous carboxylic acids containing more than 20% water, except for glyoxylic acid, which solubilised the milled wood at high water content (36%).

The ball-milled wood was completely dissolved in pyruvic acid at room temperature after 4–6 days. A shorter mixing time (1 day) was not sufficient to obtain complete dissolution (Figure 2b). In contrast, formic acid and 64% aqueous glyoxylic acid dissolved the milled wood in 4–6 h and 30–60 min, respectively. However, the three acids did not dissolve the Wiley-milled wood (20 mesh) under similar conditions, suggesting that ball-milling was necessary to disrupt wood cell walls and realise an efficient dissolution.

A strong acid (low pKa) was expected to dissolve wood more efficiently than a weak acid because trifluoroacetic acid (pKa = 0.52[21]) is known to dissolve cellulose.[22] However, glyoxylic acid (pKa = 3.18) dissolved the milled wood more rapidly than pyruvic acid (pKa = 2.39), revealing that acidity is not the only factor that determines the solubilisation efficiency.

Pyruvic, glyoxylic and formic acids contain an acyl and/or a formyl group, suggesting that these groups may contribute to their high solubilisation efficiency *via* acetal and/or hemiacetal formation. Paraformaldehyde/DMSO[23,24] and trichloroacetaldehyde (chloral)/DMF/pyridine[25–27] are known cellulose solvents and their solubilisation efficiency was attributed to the formation of hemiacetal bonds.[26]

To verify this hypothesis, methanol was reacted with the three solvents at room temperature and the products were analysed by NMR and GC–MS (Table 2). The reaction of glyoxylic acid with methanol formed 2-hydroxy-2-methoxyacetic acid (**3**) as a major product, indicating that hemiacetalisation readily occurred only by mixing glyoxylic acid and alcohol. After several days, the amount of compound **3** decreased while that of methyl 2-hydroxy-2-methoxy acetate (**6**) increased. In contrast, the formic acid reaction with methanol primarily afforded methyl formate (**5**) rather than the hemiacetal. In case of pyruvic acid, both methyl pyruvate (**1**) and methyl 2,2-dimethoxypropanoate (**2**) were detected in the reaction mixture, consistent with the occurrence of acetalisation and esterification. Consequently, the high solubilisation efficiency of pyruvic and glyoxylic acids may stem from the acetalisation and/or hemiacetalisation of formyl and acyl groups as well as the esterification of the carboxyl groups.

Table 2 Yields of products in reactions of the carboxylic acids with methanol at room temperature

Acid	Ester	Acetal or hemiacetal	Ester, acetal or hemiacetal
Pyruvic acid		N.D.[a]	
	1		**2**
	2%		6%
Glyoxylic acid	N.D.[a]		
		3	**4**
		37%	6%
Formic acid		N.D.[a]	N.D.[a]
	5		
	14%		

[a] N.D. Not detected

Although conventional high-temperature liquefaction afforded black wood solutions, the milled wood dissolution using pyruvic and glyoxylic acids as solubilising agents produced clear reddish brown solutions. This reduced blackening of these lignin solutions results from the mild, room temperature conditions. Moreover, the conversion of hydroxyl groups into the corresponding acetals and/or hemiacetals may reduce their reactivity leading to hyperdegradation.

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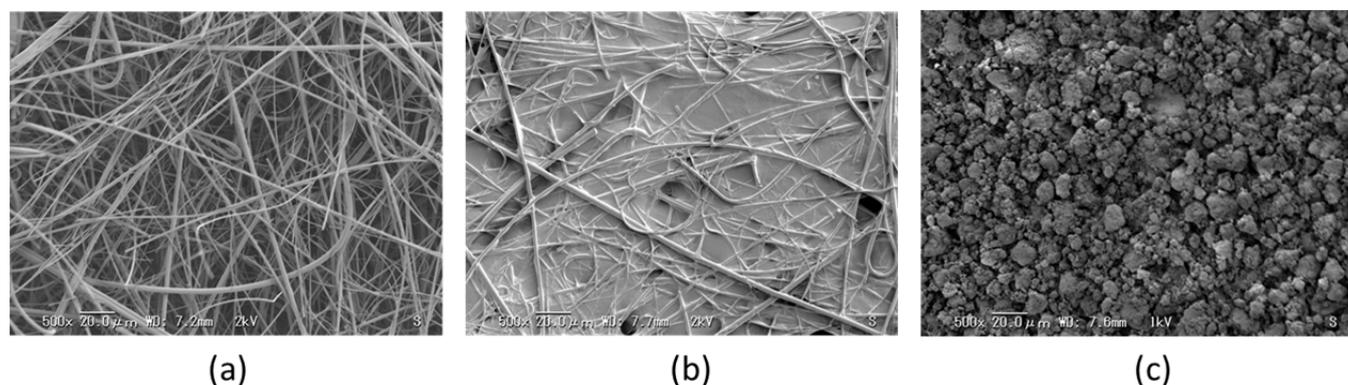
Fig. 3 SEM images of (a) filter before filtration, (b) filter after filtration of the ball-milled formic acid solution and (c) original ball-milled *E. globulus*.

Analysis of the dissolution process

To clarify the dissolution process, the time course of the amounts of filtrate and non-filterable materials was analysed. When formic acid was used as a solvent, the dissolution required a short time, as estimated from the increased viscosity 3–6 h after dissolution (Table 3, Figure 4). However, the soluble material could not be quantified by filtration after 3–6 h because of high viscosity.

After 18 h, the viscosity of the solution decreased due to hydrolysis (Figure 4), and the amounts of filtrate and non-filterable materials became measurable. The quantification supported that the milled wood was mostly dissolved in formic acid. In every measurement, the sum of filtrate and non-filterable materials exceeded 100% of the initial ball-milled wood. This clearly indicates that formic acid was bound to the wood by chemical modification such as esterification. The amounts of filtrate and non-filterable materials in pyruvic or glyoxylic acid could not be determined because the high viscosity hindered filtration. The formic acid solution was less viscous than pyruvic or glyoxylic acid solutions. However, the formic acid solution was adhesive to glass filter and the solution adhered to the filter even after fully washed with the solvent, as evident from the SEM images before and after filtration (Figure 3 (a) and (b)). This is the reason why the quantity of non-filterable fraction at 18 h and 24 h was not 0% (Table 3). None of the granular materials observed in the original ball-milled wood (Figure 3c) were detected by SEM after filtration, confirming the dissolution of ball-milled wood in formic acid.

Table 3 Effects of solubilisation time of ball-milled wood in formic acid on filtration

Time (h)[a]	Non-filterable fraction (%)	Filtrate (%)
1	92 ± 5	24 ± 3
18	16 ± 6	116 ± 33
24	14 ± 5	113 ± 5

[a] Mixtures stirred for 3–6 h were not measurable because of high viscosity.

Each 4.8% milled wood solution in carboxylic acid was more viscous than their corresponding solvent. Therefore, the dissolution process was monitored using a rotating viscometer (Figure 4). When the milled wood was dissolved in 64% aqueous glyoxylic acid (17 mPa s), the viscosity increased within 1 h and reached a value of 4170 mPa s after 1 day before decreasing. For pyruvic acid (17 mPa s), the viscosity increased to 1854 mPa s after stirring for 3 days and then decreased. For formic acid (4 mPa s), the viscosity increased to 480 mPa s after 6 h stirring before decreasing. However, the dissolution of the milled wood in pyruvic and formic acids occurred more slowly than in glyoxylic acid, which is in agreement with the changes in optical transmittance. Viscosity of solutions of cellulose derivatives depends on solvent and several solute parameters such as molecule size and substituent distribution in chemical structures. Among these factors, the differences in the type of substituents and solvents as well as degree of polymerisation significantly influence the viscosity maximum. The decrease in viscosity after reaching the maximum is explained primarily by a decrease in molecular weight upon hydrolysis after dissolution.
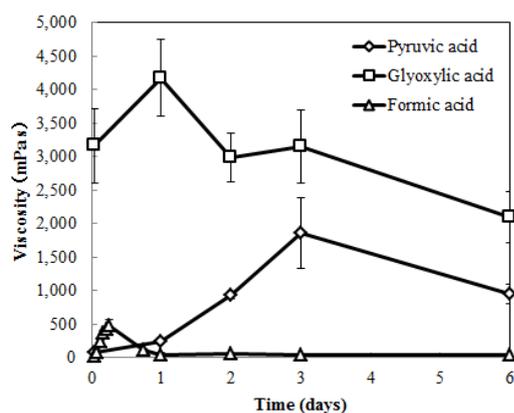
Fig. 4 Time course of viscosity changes in dissolution of ball-milled wood in pyruvic, glyoxylic and formic acids.

The optical transmittance of the 4.8% wood solution at 660 nm was measured using an optical density monitor to assess the transparency of each milled wood solution (Figure 5). The optical transmittance profile changed in agreement with the viscosity increase. Immediately after adding milled wood to carboxylic acid, optical transmittance of each acid solution was around zero because of insolubility. The transmittance increased slightly for the glyoxylic acid solution after 1 h, but remained close to zero for formic acid and pyruvic acid solutions. After 1 day, the formic acid solution became transparent with increased transmittance; however, the transmittance of the pyruvic acid solution increased more slowly because of slower dissolution. The transmittance of the formic acid solution decreased after 2 days, accompanied by a colour change to black.

With regard to colour, the transmittance of the short wavelength below 550 nm was lower than that of longer wavelength in the visible region between 400 and 750 nm (Figure 6a), resulting in reddish brown solutions. Spectrophotometer and optical density monitor gave almost the same transmittance at 660 nm. Dilute wood solutions exhibited a higher transmittance (Figure 6b) and higher transparency by visual observation (Figure 2, 0.5% solution).

These results show that viscosity and transmittance provide a good assessment of solubility and molecular weight changes after mixing.

Fig. 6 Transmittance of ball-milled wood solutions measured by spectrophotometry in wavelength scan mode: (a) formic acid, glyoxylic acid and pyruvic acid solutions (14 days) and (b) dilute solutions in formic acid (2 days).

Fluid property of acid solutions

The viscosity was measured at different viscometer rotational speeds to assess the fluid property of 4.8% wood solutions. Although this analysis is typically conducted using rheometers, viscometers can be used to determine fluid types in solutions. A fluid is classified as Newtonian if its viscosity remains constant for different rotational speeds or non-Newtonian if its viscosity varies with rotational speed.

All acid solutions exhibited shear thinning, characteristic of non-Newtonian fluids (Figure 7). Pyruvic acid (3 days), glyoxylic acid (1 day) and formic acid solutions (6 h) presented non-Newtonian indices (n) of 0.57, 0.72 and 0.41, respectively, indicative of their pseudo-plasticity (non-Newtonian index $n <$ 1) (Figure 8).

Fig. 5 Time course of optical transmittance at 660 nm for the dissolution of ball-milled wood in pyruvic, glyoxylic and formic acids.
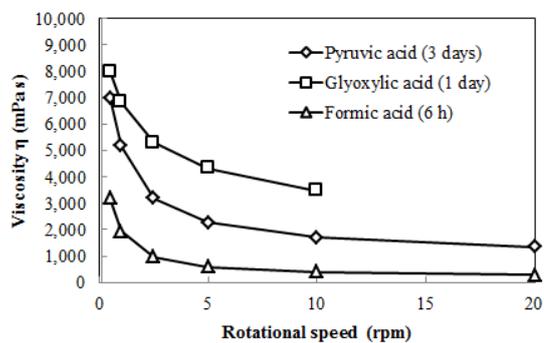
Fig. 7 Viscosity of 4.8% ball-milled wood solutions in pyruvic, glyoxylic and formic acids at various rotational speeds.
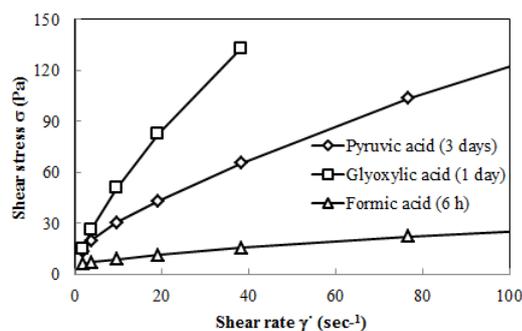
Fig. 8 Shear stress of 4.8% ball-milled wood solutions in pyruvic, glyoxylic and formic acids at various share rates converted from Fig. 7.

Polymer solutions behave as non-Newtonian fluid in many cases. Cellulose solutions have shown a non-Newtonian behaviour,[24,28,29] suggesting that the non-Newtonian nature of liquid is an evidence for the dissolution of polymers in wood. In a previous study, the n values ranged between 0.6 and 0.95 for wood pulp (degrees of polymerization (DPs) 532, 837) or cotton linter (DP 495) solutions in paraformaldehyde/DMSO and decreased (non-Newtonian) with concentration and DP.[24] The formic acid solution demonstrated a non-Newtonian behaviour ($n = 0.41$) after 6 h. However, after 6 days, its non-Newtonian index n increased to 0.96, indicating that the solution changed into a Newtonian fluid. This change is consistent with the depolymerisation of wood polysaccharides induced by hydrolysis with formic acid over a long time period.
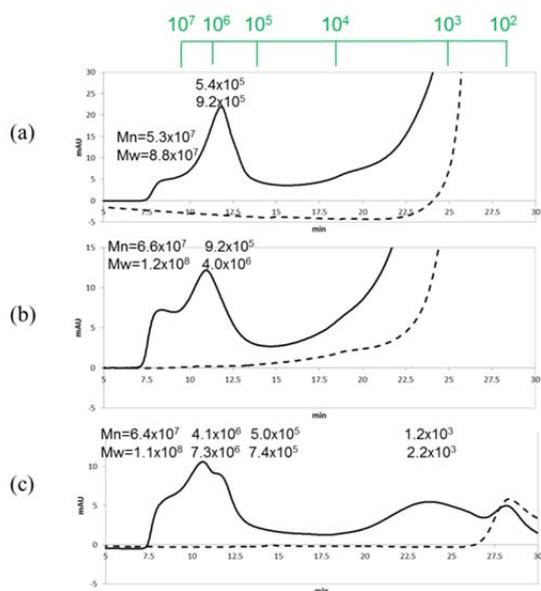
Fig. 9 SEC of ball-milled wood solutions. (a) Pyruvic acid (6 days), (b) glyoxylic acid (24 h) and (c) formic acid (24 h). The elution was monitored at 280 nm using a UV detector. The dashed lines represent the SEC of the solvents.
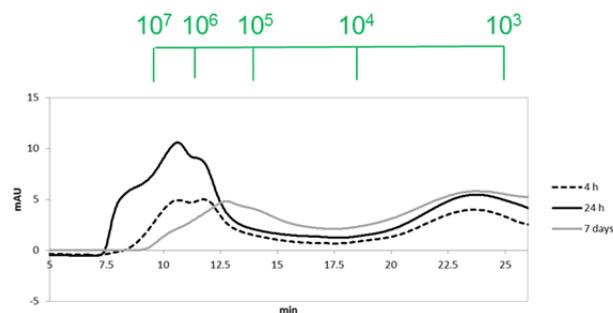
Fig. 10 SEC of ball-milled wood dissolved in formic acid after 4 h, 24 h and 7 days.

Molecular weight distributions of dissolved wood components were evaluated by SEC (Figure 9). High molecular weight regions ($Mn \geq 10^5$) presented a peak at 5.4×10^5, 9.2×10^5 and 4.1×10^6 for pyruvic acid, glyoxylic acid and formic acid solutions, respectively. The formic acid solution showed another peak at 5.0×10^5. Moreover, all the solutions showed substances with molecular weights exceeding 10^7 in the void volume region, corresponding to the elution of matrices composed of lignin and polysaccharides or their aggregates. In low molecular weight region (10^4–10^3), the formic acid solution showed a peak at 10^3, which originates from lignin–carbohydrate complexes (LCCs) or polysaccharide-free lignin. For pyruvic acid or glyoxylic acid solutions, low molecular weight compounds could not be evaluated because of an overlap with solvent absorbance peaks. Reported molecular weights amounted to 10^7–10^4, 10^5–10^4 and 10^4–10^2 for ball-milled Red Maple[4], LCC from spruce[30] and acetylated milled wood lignin from Norway spruce[31], respectively. The obtained molecular weight distributions indicate that the dissolved wood retained high molecular mass fractions exceeding Mn values of 10^5–10^3, in accordance with the previous studies.

The time course of molecular weight distributions was analysed at 4 h, 24 h and 7 days by SEC for the formic acid solution (Figure 10). SEC profiles after 4 and 24 h were similar, except for the high molecular weight intensities ($Mn > 10^5$) after 24 h. This indicates that the 24 h immersion caused a higher amount of lignin and LCCs to dissolve in the acid. After 7 days, high molecular mass components ($Mn > 10^7$) disappeared. Moreover, the absorbance intensity decreased between 5×10^5 and 5×10^6, concomitant with the formation of a new peak at 1.3×10^5. This suggests that the hydrolysis of cell wall polysaccharides induced by formic acid broke the lignin and polysaccharide matrices, separating the non-covalently linked components. The depolymerisation of LCCs may also explain this shift.

UV-visible absorption spectra of milled wood solutions were measured. Because pyruvic acid or glyoxylic acid showed strong absorption below 400 nm, the absorption spectra were recorded for formic acid solutions, which absorb UV light below 260 nm (Figure 11). Spectra obtained at two different concentrations at 0.048% and 0.024% showed a maximum at 280 nm. Although furfural derivatives present an absorption peak at 280 nm, this peak is ascribed to lignin because carbohydrates are stable at ambient temperature. The reported absorption coefficient of lignin at 280 nm amounted to 20 $Lg^{-1}cm^{-1}$ (Acetyl bromide, soft wood and hard wood),[32] 19 $Lg^{-1}cm^{-1}$ (3% H_2SO_4, spruce SEW)[33] and 17.5 $Lg^{-1}cm^{-1}$ (lignosulphonate).[34] Using an absorption coefficient of 19 $Lg^{-1}cm^{-1}$ and a specific gravity of 1.22 for formic acid, the lignin content of milled wood was calculated to be ca. 15%–

16%. These results confirm that the milled wood was dissolved into the acid.
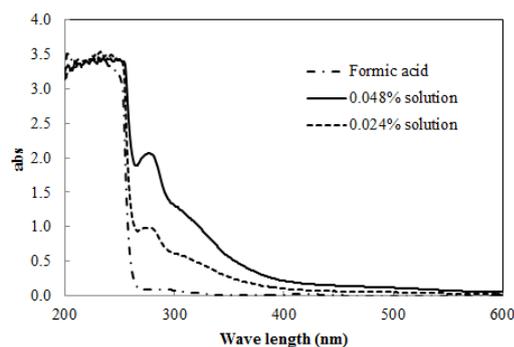
Fig. 11 UV absorption of the dilute milled wood solution in formic acid.

Transparent film formation

A transparent brown film was obtained by evaporation of the formic acid solution without fractionation (Figure 12). This transparency confirms that the ball-milled wood was actually dissolved in formic acid.
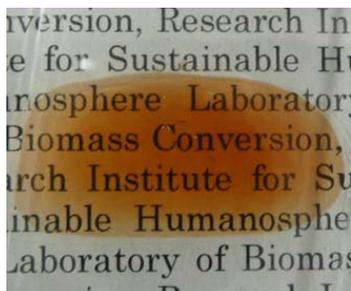
Fig. 12 Photograph of the film formed after evaporation of the ball-milled wood solution in formic acid

Precipitation in ether solvents and fractionation

The separation of wood components from wood solution is important for their conversion and utilization. Because the dissolution of whole wood was performed at room temperature, the fractionation of the solutions by precipitation will lead to an environmentally benign separation of wood components without intensive modification of the native structures.
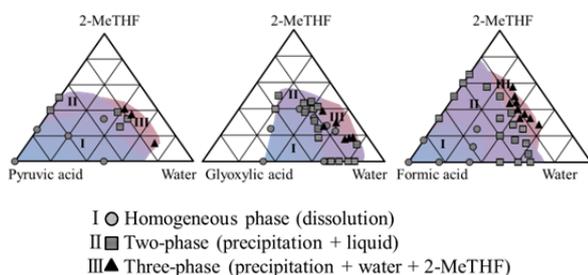
Fig. 13 Phase diagram of ternary mixture of ball-milled wood solution in acid, 2-MeTHF and water.

Fig. 14 Photograph showing three phase-separated layers (III) formed by addition of 2-MeTHF and water to the ball-milled wood solution in formic acid.

The separation process relies on the addition of a water-immiscible solvent that is stable against the acid used, justifying the choice of 2-MeTHF, a furan derivative available from biomass. The effect of 2-MeTHF and water addition to the wood solution was analysed in detail at various relative compositions (Figure 13). The balled-milled wood solution remained homogeneous upon addition of water and 2-MeTHF at weight ratios below 10% (I). However, at weight ratios exceeding 50:50 (w/w), precipitates formed in most cases upon addition of water and 2-MeTHF. Although 2-MeTHF and water are immiscible, mixing the solvents with acids resulted in homogeneous solution at certain compositions (Figure 13 (II)). When acid, water and 2-MeTHF were mixed at a weight ratio of around 20:40:40, water and 2-MeTHF layers were separated and precipitates formed (III) (Figure 14).

In the case of mixture III, the 2-MeTHF layer was isolated and the aqueous suspension was filtered to provide a whitish, light brown solid. Water-miscible dioxane and DMSO have been used as lignin solvents but have caused coloured substances to adhere to solids during fractionation. A water-immiscible solvent can dissolve these substances in the organic phase to separate them from the solid. Although a poor solvent for these coloured substances, 2-MeTHF dissolved them when mixed with acid.

The fractionation of the ball-milled wood solution in formic acid provided three portions, Fr. 1 (precipitation), Fr. 2 (aqueous layer) and Fr. 3 (2-MeTHF layer), using solvent system III (formic acid, water and 2-MeTHF, 20:40:40, w/w/w) (Table 4). The fractionation was conducted 1 day (No. 1) and 8 months (No. 2) after dissolution. The total recovered weight exceeded 100% because a portion of formic acid was bound to the wood components. A long immersion period increased the total weight because the acid was incorporated into the wood components.

Table 4 Yields (%) of fractions isolated from a ball-milled wood solution in formic acid using 2-MeTHF and water

No.	Time of separation after dissolution	Original milled wood	Fr. 1 Precipitate	Fr. 2 Aqueous layer	Fr. 3 2-MeTHF layer
1	1 day	100	73	19	18
2	8 months	100	64	37	33

Analysis of wood fractions

Fractions isolated by addition of 2-MeTHF and water to the ball-milled wood solution in formic acid were characterised by Py–GC–MS (Figure 15) and compositional analysis. Area ratios

of compounds detected by Py–GC–MS are shown in S1. Levoglucosan (compound No. 30), a product from cellulose pyrolysis, was the main component of the precipitate (Fr. 1). The 2-MeTHF layer (Fr. 3) primarily contained lignin-derived substances (compound No. 21 etc.). When the fractionation was performed 1 day after dissolution (No. 1), compounds derived from lignin and hemicelluloses were found in the precipitate (Fr. 1) and a small portion of cellulose-derived substances were detected in the 2-MeTHF layer (Fr. 3). We also fractionated the solution after 3 and 7 days. The components were almost the same as those of the 1 day sample. However, when the wood solution was fractionated 8 months after dissolution (No. 2), a small amount of lignin-derived substances was present in the precipitate and the 2-MeTHF layer contained an increased amount of cellulose-derived substances. This demonstrates that a long immersion decomposed lignin- and cellulose-derived substances and solubilised them by acid-enabled chemical modification. The water layer (Fr. 2) contained hemicellulose-derived furfural (compound No. 2), cellulose-derived levoglucosan and lignin-derived substances. Low molecular weight compounds were assumed to dissolve in water.

The compositional analysis revealed that the precipitate obtained after 1 day contained a higher holocellulose amount and a lower lignin amount than the original wood (Table 5), consistent with Py–GC–MS results. Neutral sugar analysis indicated that this precipitate contained a higher glucose concentration and a lower xylose concentration than the original wood. Therefore, the precipitate obtained from the wood solution using 2-MeTHF and water mainly consists of cellulose as well as hemicelluloses and lignin at lower concentrations than in the original wood.
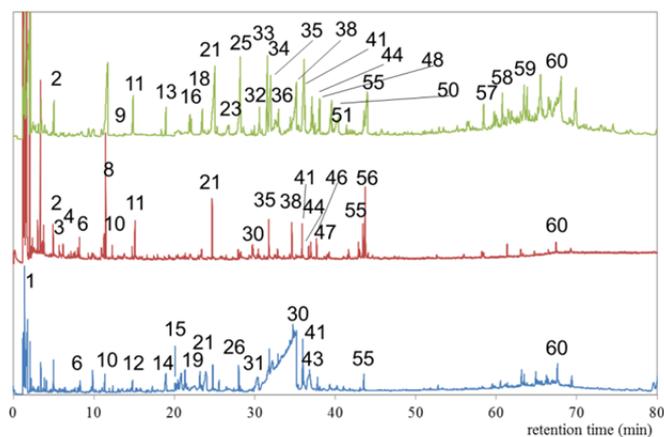
Table 5 Compositions (wt%) of the ball-milled wood and solid fraction obtained by 2-MeTHF/water fractionation of the formic acid solution at room temperature

		Ball-milled *E. globulus*	No. 1–Fr. 1[a]
Holocellulose		63.1	77.7
Klason lignin		20.3	14.0
Acid soluble lignin		2.3	2.8
Carbohydrates	Ara	0.0	0.0
	Xyl	13.9	7.9
	Man	0.0	0.0
	Gal	0.0	0.0
	Glc	45.6	58.7

[a] Precipitate isolated from the ball-milled wood solution in formic acid using 2-MeTHF and water (Fig. 1).

The ball-milled wood and the cellulosic precipitate (Fr. 1) obtained from the formic acid solution by 2-MeTHF/water fractionation were examined by SEM. The precipitate (Fr. 1) comprised a porous material containing small pores with diameters of up to *ca.* 10 μm (Figure 16). Precipitates from cellulose–ionic liquid solution have been reported to have porous structures.[35] These morphological features confirm that the ball-milled wood was actually dissolved in formic acid and then precipitated by addition of 2-MeTHF and water. A transparent film exhibiting a high lignin content was obtained by evaporation of the wood solution and a porous solid was obtained by reprecipitation during the fractionation. This demonstrates that the wood solution can produce different solid forms by various post-treatment processes.

The dissolution of whole wood in ionic liquid and other solvents such as DMSO have been studied extensively over this decade. However, its dissolution and fractionation using simple solvents without any additives at room temperature have been difficult to achieve. Our method offers a process that solubilizes and separates whole wood using simple α-keto acid or aldehydic carboxylic acid, in combination with precipitation using 2-MeTHF and water at room temperature. The water-immiscible solvent used for separation can be recovered from the wood by simple partitioning. This α-keto acid and aldehydic carboxylic acid system is expected to find wide applications in solution reactions of wood components and liquid fuels. It may also be implemented in the production of polymer support for adsorption and gradual release of organic compounds by chemical and enzymatic modification.

Fig. 15 Py–GC–MS total ion chromatograms of each portion obtained by 2-MeTHF/water fractionation of a ball-milled wood solution in formic acid after 1 day (No. 1).
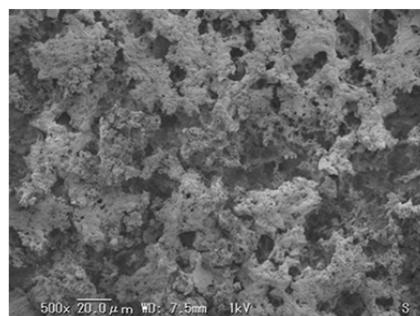
Fig. 16 SEM image of precipitate (Fr. 1) obtained by 2-MeTHF/water fractionation of the wood solution in formic acid.

Conclusions

The dissolution of whole wood has attracted major research efforts because of it impacts wood component separation and the biological and chemical modification of these components in solution. The wood solution can be used also as a renewable liquid fuels if the solvent is cheap enough for energy recovery by burning. In this study, ball-milled wood dissolved in pyruvic, glyoxylic and formic acids at room temperature. The simplest α-keto acid, pyruvic acid, is an important intermediate in several metabolic pathways such as glycolysis, gluconeogenesis, TCA cycle and amino acid and fatty acid biosyntheses. The use of this safe and highly biodegradable α-keto acid as a wood solvent opens the door to new applications related to lignocellulosic biorefinery processes. The simplest α-formyl acid, glyoxylic acid, is the key metabolite in the glyoxylate path in microorganisms and plants. Biological production of this acid can be achieved by blocking the transformation of glyoxylate into malate in this path. The simplest fatty acid, formic acid, has been used in various chemical industries for its economic advantages. Moreover, this solvent has a low boiling point that facilitates its recovery from reactive media containing wood and its degradation products. Here, a method was also developed to fractionate the wood solution using the water-immiscible solvent 2-MeTHF. Because the dissolution and separation were achieved at room temperature with simple compounds derivable from biomass, our results are expected to lead to self-sufficient biorefinery systems that transform wood components to chemicals, fuels and functional polymers.

Notes and references

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