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Materials and Methods

Sample preparations

Chemicals.  Deionized water, purified using a Milli-Q® Advantage A10® system (Millipore™, Eschborn, Germany), was used. Other reagent-grade chemicals were purchased from Tokyo Kasei Chemicals, Wako Pure Chemical Industries or DuPont™ Genencor® Science and used as received.

A cedar, white birch, and rice straw were powdered (10 µm) by dry ground jet milling for several seconds. L-MODU is gift from Idemitsu Kosan Co., Ltd (S901, $M_n = 130,000$, Softening Point = 120 °C). Poly(propylene) is gift from Mitsubishi Chemical
Co. (SA06, Softening Point = 180 °C). Poly(L-lactic acid) is gift from UNITIKA Ltd. ($M_w = 140,000$, D ratio = 1.4%, Softening Point = 170 °C).

**Constituent separation of polysaccharides and lignin from plants.** For simultaneous physical comminution and enzymatic saccharification, we grinded the mixture of 50 g of degreased plant powder, 10 g of an equivalent mixture of OPTIMASH XL (containing of cellulase and xylanase) and OPTIMASH BG (containing of xylanase and β-glucosidase), and 440 g of 100 mM phosphate buffer (pH = 5.0) by bead milling (stermill® LMZ015; Ashizawa Finetech Ltd., Japan) equipped with zirconia beads (0.5 mm$\phi$) with 14.0 m/s of peripheral velocity at 40 °C. For prevention of damage to enzyme, inner wall of vessel in LMZ015 was covered by ceramic (The enzymes were inactivated under the beads milling in vessel with the stainless steel wall.). After 2 h of bead milling, the obtained mixture was centrifuged at 10,000 ×g for 30 min to obtain a supernatant and precipitate of the mixture. The obtained precipitate was mixed with an equivalent phosphate buffer and 5 g of an equivalent mixture of OPTIMASH XL and OPTIMASH BG, followed by additional beads milling at same condition above for one more time. After two times of beads milling, an additional saccharification of the sample without beads milling was performed at 50 °C for 48 h. Then, the supernatant and precipitate were collected by centrifugation at 10,000 ×g for 30 min as a saccharide solution and lignin slurry, respectively. The lignin slurry was washed twice with an equal amount of ultra-pure water using centrifugation at 10,000 ×g for 30 min.

**Ethanol fermentation of saccharide solution.** All of supernatant after centrifugation until the whole separation procedures (i.e., sugar solution) was used for ethanol
fermentation. A YMbroth that contained 0.1% yeast extract, 0.0025% MgSO$_4$·7H$_2$O, and 0.05% (NH$_4$)$_2$HPO$_4$ was used as preculture media for the *Saccharomyces cerevisiae* NBRC 2347. *S. cerevisiae* NBRC 2347 was precultured for 3 days at 30 °C. Then, precultured cells were dispersed in the saccharide solution (1.0 g per 50 mL of solution) and stirred at 32 °C for 72 h.

**Acetylation of lignin in the slurry.** The lignin slurry (30 mL) was mixed with 70 mL of pyridine. After removal of water as an azeotrope with pyridine, 5 mL of acetic anhydride and 10 mg of 4-(N,N-dimethylamino)pyridine were mixed with the sample. The mixture was stirred at 50 °C for 12 h. The products were purified by reprecipitation from cold water. The products were collected *via* vacuum filtration on a 100 nm Millipore filter and dried *in vacuo* at r.t. for 6 h. The acetylated lignin can be dissolved with deuterated dimethyl sulfoxide (DMSO-$d_6$) for NMR measurements.

**Mixing of lignin powder with various polymers.** A certain amount of lignin powder and polymer (L-MODU, poly(propylene), and poly(l-lactic acid)) were mixed and kneaded by an injection molding machine (IMC-18D7, Imoto Machinery Co., Ltd. Japan). The obtained pellets were hot-pressed at 3~5 MPa for 10 min under heating (temperature is shown in Figure caption).

**Measurement**

**Dynamic light scattering (DLS).** DLS measurement of diluted slurries (equal to approx. 1.0 wt/v%) was performed using a Zetasizer Nano ZS equipped with a 4 mW He–Ne laser (wavelength = 632 nm) (Malvern Instruments Ltd., United Kingdom).
Viscosity measurement. Viscosity of the sample was measured by a viscometer (DV2T, Brookfield Asset Management Inc. USA).

High performance liquid chromatography (HPLC). A saccharide solution and a fermented solution were filtered through a syringe filter with a Nylon membrane (porosity, 0.2 μm) prior to injection into the HPLC column. Resulting samples were analyzed by HPLC (LC-20AD, Shimadzu Co. Ltd. Japan) on a combination of ligand exchange and size exclusion chromatography column (Sugar KS-802, 6 μm column, 8.0 × 300 mm, Shodex Co. Ltd. Japan) at 80 °C with water flowing at 1 mL/min. The eluting sugar compounds and ethanol present in a sample were detected using an RI detector (RI-201H, Shodex Co. Ltd. Japan) and identified by comparing their retention times with those of standard purified compounds.

Fourier transform infrared (FT-IR) spectrum. FT-IR spectrum was recorded on a JASCO FT/IR-4100 spectrometer using KBr pellets of lignin powder.

Nuclear magnetic resonance (NMR) spectroscopy. $^1$H-NMR spectrum was measured on a JEOL AL500 MHz spectrometer at 20 °C. Chemical shifts are reported in ppm downfield from SiMe₄, using the solvent's residual signal as an internal reference.

Thermogravimetric analysis (TGA). TGA was carried out on a Rigaku Thermo plus TG8129 under nitrogen flow at a scanning rate of 10 °C min⁻¹.

Measurements of tensile stress–strain characteristic. Tensile stress–strain measurements of self-standing films were performed using a Tensilon Universal Tester (Tensilon RTC-1250, Orientec Co.) at 25 °C. The sample strip was 2 mm wide, 20 mm long, and typically 0.1 mm thick. A 5 mm inner position of each long end of the sample was
clamped using a polyimide tape covering to prevent the sample from slipping out of the holder. The tensile stress–strain response was measured using a load cell (UR-10L-A, Orientec Co.) at 100 mm min$^{-1}$ head speed. Young's modulus and tensile fracture strength were estimated by averaging the values for three samples. The Young's modulus was determined from the averaged slope over the range 0–0.01 of the strain ratio from the stress–strain curve.
Fig. S1 Schematic illustration of the experimental procedures for wet-type bead milling of plants without enzymatic saccharification. Until bead milling, the plant powders were crushed by zirconia beads. After the second bead milling (2.0 h × 2), the slurry-like mixture is obtained (polysaccharide & lignin). The diameter of lignocellulose particle was polydispersed between several hundred nm to thousand nm; that is approx. ten times larger than that of lignin particles obtained by wet-type bead milling of plants with enzymatic saccharification (38 nm; Fig. 2). The drying of the obtained mixture gave pale-brown colored inhomogeneous films.
**Fig. S2** Saccharification ratios of cedar powder comminuted under various conditions.

Except for our method (1x and 2x beads milled), the wood powders obtained by various preprocessing (i.e., chopping, several seconds of jet milling, and 48 hours of vibration milling) were enzymatically saccharified in phosphate buffer *without beads milling*. The numbers above each column are the saccharification ratios, which are based on the percentage of cellulose converted to glucose and disaccharide. Sugars were analyzed by HPLC.
Fig. S3 FT-IR spectrum of lignin powder. The followed peaks as similar with previous studies\textsuperscript{S3-S5} were recognized in this spectrum;

1; 1725 cm\textsuperscript{-1} (unconjugated carbonyl groups), 2; 1657 cm\textsuperscript{-1} (conjugated carbonyl groups), 3; 1601 cm\textsuperscript{-1} (aromatic groups and carbonyl groups), 4; 1509 cm\textsuperscript{-1} (aromatic groups), 5; 1463 cm\textsuperscript{-1} (methyl groups), 6; 1422 cm\textsuperscript{-1} (aromatic groups), 7; 1368 cm\textsuperscript{-1} (hydroxyl groups in aromatic ring or methyl groups of polysaccharides), 8; 1330 cm\textsuperscript{-1} (aromatic groups), 9; 1265 cm\textsuperscript{-1} (hydroxyl groups in guaiacyl methoxyl structure), 10; 1215 cm\textsuperscript{-1} (C-C, C-O, and C=O groups), 11; 1120 cm\textsuperscript{-1} (methyl groups in syringyl-type aromatic ring), 12; 1082 cm\textsuperscript{-1} (methyl groups in guaiacyl-type aromatic ring or carbon-oxygen bonding of polysaccharides), 13; 1030 cm\textsuperscript{-1} (methyl groups in guaiacyl-type aromatic ring), 14; 856 cm\textsuperscript{-1} (methyl groups in syringyl-type aromatic ring), 15; 815 cm\textsuperscript{-1} (methyl groups in aromatic ring).
Fig. S4 $^1$H-NMR spectrum of acetylated DMSO-$d_6$ solution. It is considered that the doublet peak at 1.94 and 1.90 ppm originated from the acetyl-CH$_3$ groups, indicating that these peaks also support the progress of acetylation. Furthermore, the peaks of guaiacyl-type aromatic groups (7.00 ppm) and of β-O-4 /β-1 bonding (5.93 ppm) were recognized. Other tendencies of the spectrum were also similar in profile to that of acetylated milled wood lignin$^{86-88}$ that verifies the presence of non-denatured lignin in the bead milled slurry of plant powder.
Photographic images of self-standing composite films consisting of lignin and L-MODU (a), poly(propylene) (b), and poly(\(L\)-lactic acid) (c) with a 60 w/v\% ratio of lignin. The kneading temperature of the mixture of lignin and L-MODU, poly(propylene), and poly(\(L\)-lactic acid) is 130, 220, and 200 °C, respectively, depending on the melting temperature of polymer. Due to the high mixing temperature, the composite film consisting of poly(propylene) and poly(\(L\)-lactic acid) exhibited a dark brown color that may have emerged from char formation by residual polysaccharides.
**Fig. S6** TG-DTA result for a composite film consisting of nano-particulate lignin and L-MODU with a ratio of 80 w/v% of lignin (red), nano-particulate lignin (green), and L-MODU itself (blue). The 5% decomposition temperature ($T_{d5}$) of the composite film, lignin, and L-MODU is 224, 180, and 356 °C, respectively. Furthermore, the residue of lignin and composite film after heating at 500 °C is 40 w/v% and 18 w/v%, respectively.
References


