

Supporting Information for:

**A Small Amount of Nanoparticulated Plant
Biomass, Lignin, Enhances Heat Tolerance for
Poly(ethylene carbonate)**

Kazuhiro Shikinaka,* Haruka Soutome, Yuki Kubota, Yoichi Tominaga,* Masaya Nakamura, Ronald R. Navarro, and Yuichiro Otsuka

Experimental Section

Materials: Ultrapure water was employed throughout the study and was obtained using a Milli-Q® Advantage A10® system (Millipore™, Eschborn, Germany). Poly(ethylene carbonate) ($M_n = 39,000$) was purchased from Empower Materials, Inc (New Castle, USA). Other reagent-grade chemicals were purchased from Tokyo Kasei Chemicals, Wako Pure Chemical Industries, or DuPont™ Genencor® Science and were used as received. Cedar wood was powdered ($10\ \mu\text{m}\phi$) by dry ground jet milling for several seconds.

Simultaneous enzymatic saccharification and comminution (SESC) of plant:^{S1} For SESC treatment, a mixture of degreased plant powder (50 g), OPTIMASH XL (containing cellulase and xylanase) and OPTIMASH BG (containing xylanase and β -glucosidase) (1:1 ratio, 10 g), and 100 mM phosphate buffer (pH 5.0, 440 g) was ground by bead milling (Labstar® LMZ015; Ashizawa Finetech Ltd., Japan) at a peripheral velocity of 14.0 m/s at 50 °C. To prevent damage to the enzyme, the inner wall of the LMZ015 vessel was covered with a ceramic lining (stainless steel inactivates the enzyme). Following bead milling for 2 h using 0.5 mm ϕ zirconia beads, the obtained mixture was centrifuged at 10,000 \times g for 30 min. The saccharide-containing supernatant was then collected, and the precipitate was milled once more under the same conditions and buffer strength, but using 5 g of the enzyme mixture and smaller zirconia beads (0.1 mm ϕ). Following this second milling process, additional saccharification of the generated slurry was performed at 50 °C for 48 h. The final slurry was centrifuged at 10,000 \times g for 30 min, after which the supernatant was recovered, and the lignin-rich precipitate was washed twice by mixing it with an equal amount of ultrapure water prior to centrifugation under the above-described conditions.

Preparation of non-deteriorated lignin and PEC composites: Defined amounts of powdered non-deteriorated lignin and melted PEC pellets were mixed and kneaded at 120 °C by an injection molding machine (IMC-A300, Imoto Machinery Co., Ltd. Japan). The obtained composites were hot-pressed at 3–5 MPa for 10 min at 120 °C to mold into films.

Thermogravimetric analysis: Thermogravimetric analysis was carried out on a TG/DTA7200 apparatus (Hirachi High Technology Corp., Japan) under nitrogen flow with temperature increasing at a rate of 10 °C min⁻¹ or with temperature holding at 180 °C.

Transmission electron microscopy (TEM): TEM observation of composite films were performed for their stained cross sections. The preparation of cross sections and their TEM observation were prepared as follows;

The composite films (PEC : non-deteriorated lignin = 8 : 2 (w/w%)) were stained with 4% osmium tetroxide (OsO_4) solution (TAAB Co., UK) and then were embedded to Quetol-651 resin (Nisshin EM Co., Tokyo). The embedded samples were cut into slices approximately to thickness 70 nm using an cryo ultramicrotome at $-100\text{ }^\circ\text{C}$ (EM UC7; Leica Microsystems, Germany). These sections were collected on a 100-mesh Cu grid with and elastic carbon membrane (Nisshin EM Co.). TEM images were obtained by a JEM-1400 (JEOL Ltd., Japan) at an acceleration voltage of 120 kV. The digital TEM data were obtained using a slow-scan CCD camera (Gatan USC1000, Gatan Inc.) and converted into images with a frame size of 1024×1024 pixels. The non-deteriorated lignin in PEC was confirmed as black objects because of preferential staining of OsO_4 to numerous carbon double bonds in precursors of lignin.^{S2}

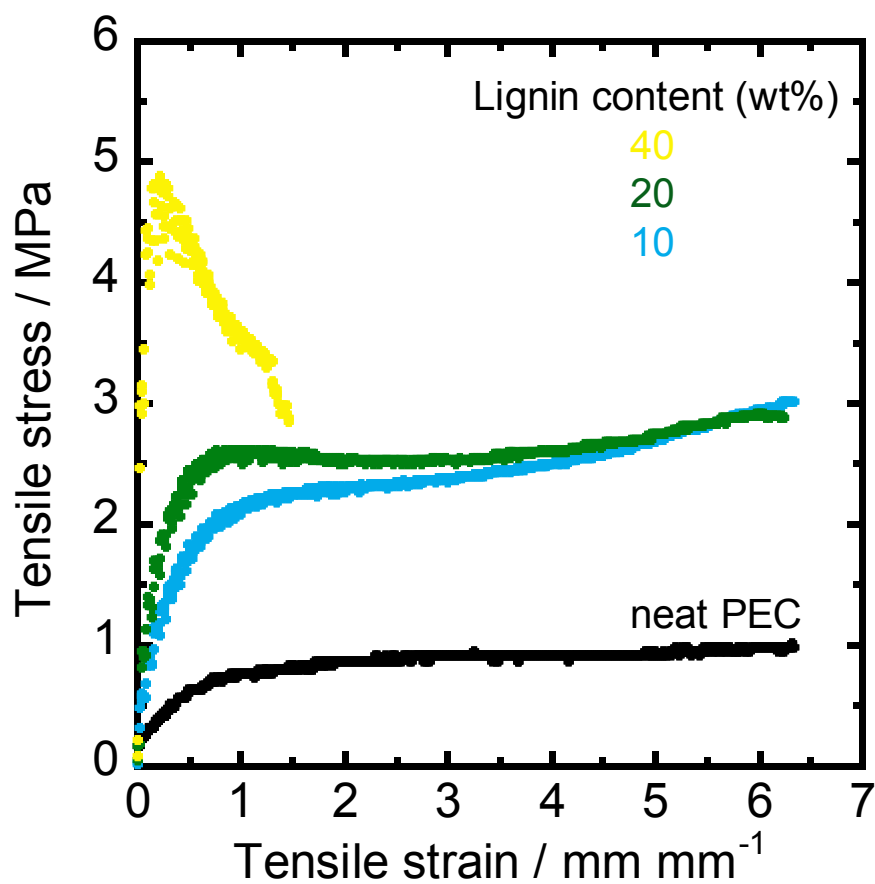


Fig. S1. Typical tensile stress-strain curves of composite films consisting of PEC and non-deteriorated lignin at various composition ratios. Tensile stress-strain measurements of composite films were performed using an OZ502 system (Sentec) at 25 °C. The dumbbell-shaped sample (2 mm inner width, 14.5 mm gauge length, and typically 0.1 mm thick) were prepared by a cutting machine (Dumb Bell Co., Ltd). The tensile stress-strain response was measured using a load cell at 7 mm min⁻¹ head speed.

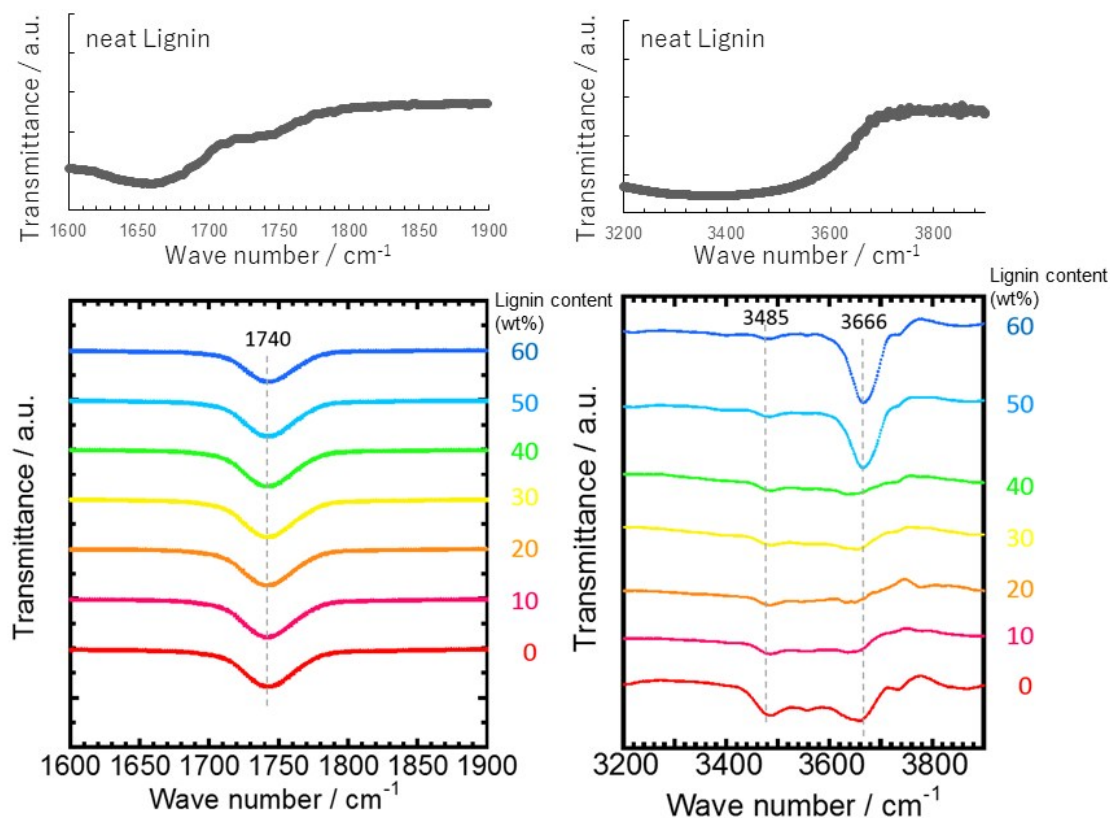


Fig. S2. FT-IR spectra of composite films consisting of PEC and non-deteriorated lignin at various composition ratios. The peaks at 1740, 3485, and 3666 cm^{-1} , which originate from the vibration of the C=O groups and OH groups in the PEC and lignin, never shift with the changing composition ratio (These peaks are never recognized in the spectrum of neat lignin). These spectra indicate an absence of hydrogen bonding between PEC and non-deteriorated lignin. FT-IR spectra were recorded on a JASCO FT/IR-4100 spectrometer using composite films and by using the attenuated total reflection method (for composite films) or the KBr method (for neat lignin).

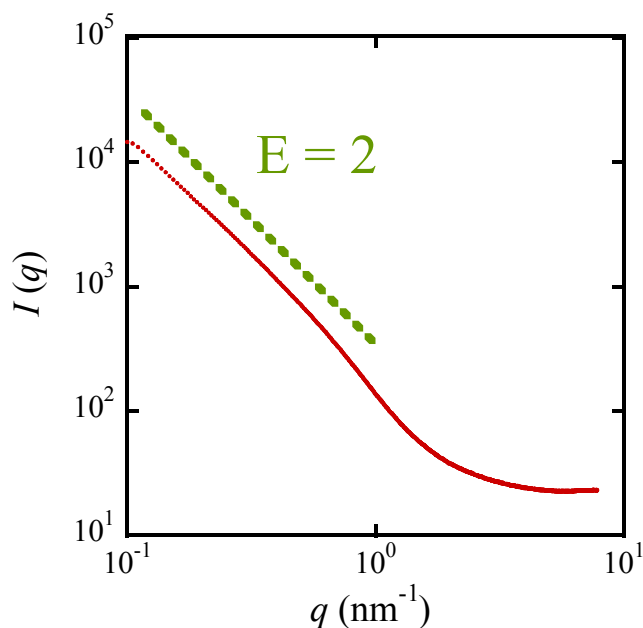


Figure S3. X-ray scattering curve of the non-deteriorated lignin solution. E was estimated from the slope of the tangent (green dotted line), where the slope of the curve, E , in the relationship $I(q) \sim q^{-E}$, where $I(q)$ is the scattered intensity and q is the scattering vector (in nm^{-1}), indicates that the fractal dimension of the molecular shape,^{S3,S4} is 2.0 at $q = 0.10\text{--}0.85 \text{ nm}^{-1}$. In addition, no dependence was observed at high q values (*i.e.*, $E = 0$ at $q > 0.85 \text{ nm}^{-1}$), which indicates the absence of a periodic structure across the corresponding q range. Thus, the E value at $q = 0.10\text{--}0.85 \text{ nm}^{-1}$ indicates that non-deteriorated lignin behaves as a platelet-shaped particle. The non-deteriorated lignin solution (a clear, brown-colored solution) was obtained by mixing 6.8 wt/v% of lignin colloidal dispersion with aqueous sodium hydroxide to a final concentration of 0.025 mol/L. The X-ray scattering experiments were performed as follows: Synchrotron X-ray scattering experiments were performed using the SPring-8 (Hyogo, Japan) synchrotron orbital radiation beam line BL40B2, which has a double-crystal silicon monochromator and focusing mirrors held at 25 °C. The wavelength of the X-rays was 0.10 nm, and the beam size was 0.2 mm ϕ at the detector position. All X-ray scattering experiments were performed by introducing the alkaline solution of non-deteriorated lignin into a cylindrical quartz capillary (2.0 mm ϕ , wall thickness 0.01 mm; Hilgenberg GmbH, Germany) at 25 °C. Images of the scattering patterns were obtained using an imaging plate with a frame size of 3000 \times 3000 pixels and a pixel size of 100 \times 100 μm (RIGAKU R-AXIS VII). The specimen-to-detector distance was maintained at 1.16 m, the sample exposure time was 10 s, and the photon flux of the X-ray source was approximately $2 \times 10^{10} \text{ photon s}^{-1} \text{ mm}^{-2}$. We confirmed that

denaturation of the lignin solution did not occur, even after continuous synchrotron X-ray irradiation for 30 s, and therefore, we judged that little radiation damage occurred during our experiments. The data were corrected by background scattering from pure water alone, and the two-dimensional scattering patterns were circularly integrated and converted into a one-dimensional format (denoted as the scattering curve) by using FIT2D software.

References

S1 K. Shikinaka, Y. Otsuka, R. R. Navarro, M. Nakamura, T. Shimokawa, M. Nojiri, R. Tanigawa and K. Shigehara, *Green Chem.*, 2016, **18**, 5962.

S2 D. Kai, M. J. Tan, P. L. Chee, Y. K. Chua, Y. L. Yap and X. J. Loh, *Green Chem.*, 2016, **18**, 1175.

S3 G. Porod, *Small-angle X-ray scattering*, ed. O. Glatter and O. Kratky, Academic Press, London, UK, 1982.

S4 P. W. Schmidt, *The Fractal Approach to Heterogeneous Chemistry*, John Wiley & Sons, New York, USA, 1989.