Utilization of Lignocellulosic Biomass via Novel Sustainable Process

Kazuhiro Shikinaka\textsuperscript{1,*}, Yuichiro Otsuka\textsuperscript{2}, Masaya Nakamura\textsuperscript{2}, Eiji Masai\textsuperscript{3}, and Yoshihiro Katayama\textsuperscript{4}

\textsuperscript{1} Research Institute for Chemical Process Technology, National Institute of Advanced Science and Technology (AIST), Nigatake, 4-2-1, Miyagino-ku, Sendai, Miyagi 983-8551, JAPAN
\textsuperscript{2} Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, JAPAN
\textsuperscript{3} Department of Bioengineering, Nagaoka University of Technology, Nagaoka, Niigata 940-2188, JAPAN
\textsuperscript{4} College of Bioresource Technology, Nihon University, Fujisawa, Kanagawa 252-0880, JAPAN

Abstract: In this review, we show novel methods for utilizing lignocellulosic biomass, polysaccharides, and lignin. Firstly, the simultaneous enzymatic saccharification and comminution (SESC) of plant materials is described as an extraction method for lignocellulosic biomass that does not require toxic reagents or organic solvents. Secondly, we demonstrate the material utilization of non-deteriorated lignocellulosic biomass extracted by SESC, such as for sugar and ethanol synthesis, and as a heatproof filler. Finally, we exhibit the use of a functional monomer (e.g., in disinfection chemicals, cesium chelation, and building blocks for polymers), 2-pyrone-4,6-dicarboxylic acid, derived from lignin via metabolic degradation.

Key words: lignocellulosic biomass, polysaccharide, lignin, heatproof filler, simultaneous enzymatic saccharification and comminution, 2-pyrone-4,6-dicarboxylic acid

1 Introduction
1.1 Recent progress in utilization of lignocellulosic biomass via physicochemical extraction

To achieve sustainable social development, breaking away from petroleum-based material science is essential\textsuperscript{1–17}. As a natural and renewable alternative to petroleum-based resources such as polylactide\textsuperscript{3}, waste plant biomass has attracted attention with respect to generating energy and functional materials\textsuperscript{2, 4–7}. Lignocellulosic biomass is the main component of waste plant biomass, consisting of polysaccharides and lignin. Polysaccharides, including cellulose and hemicellulose, have been used as pulp\textsuperscript{1}, biofuel\textsuperscript{11, 12}, and nanofibrous materials\textsuperscript{13}. Lignin, an aromatic polymer consisting of propenyl phenol units, also has potential as a renewable alternative to synthetic aromatic polymers such as polyimide\textsuperscript{14}. Several studies examined the potential uses of lignin\textsuperscript{15}, e.g., in high-performance thermoplastics\textsuperscript{8}, phenolic resin\textsuperscript{16}, and composite film\textsuperscript{17}.

Unfortunately, cellulose, hemicellulose, and lignin in plants are chemically linked in a complex manner and are thus difficult to separate efficiently\textsuperscript{18}. The most reliable method for extracting these compounds requires strong chemicals such as acids, bases, and organic solvents under high temperature and pressure\textsuperscript{19–21}. These conditions for physico-biological extraction are considered necessary to disrupt the macroscopic cell wall structure and break the interactions between lignin and polysaccharides\textsuperscript{22}. However, they also cause deterioration and induce side reactions that prevent full utilization of the extracted compounds for biofuel and other secondary products. Such technologies for lignocellulosic processing remain to be successfully implemented on a commercial scale\textsuperscript{23, 24}. To overcome these challenges, much effort has been devoted to developing new approaches to extract lignocellulosic biomass, such as glycerol thermal processing\textsuperscript{25–27}, γ-valerolactone-based processes\textsuperscript{20}, and sulfite pretreatment to overcome the recalcitrance of lignocellulose (SPORL)\textsuperscript{28}.

*Correspondence to: Kazuhiro Shikinaka, Research Institute for Chemical Process Technology, National Institute of Advanced Science and Technology (AIST), Nigatake, 4-2-1, Miyagino-ku, Sendai, Miyagi 983-8551, JAPAN
E-mail: kaz.shikinaka@aitst.go.jp (K.S.).
Accepted June 3, 2018 (received for review April 25, 2018)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jocs
1.2 Physical comminution of plants—a candidate for lignocellulosic biomass extraction via a sustainable process

Physical comminution is an important step in preparing lignocellulosic waste for producing biofuel\(^{29-31}\). Compared to chemical processes, much less progress has been made in this technique as the only pretreatment prior to enzymatic saccharification, and this relates to its inability to separate lignin and polysaccharide\(^{32}\). This problem is increased when working with woody biomass\(^{33}\). Though several types of physical comminution that do not involve chemicals and heat energy were evaluated for processing agricultural wastes such as bagasse with promising results\(^{33}\), the higher lignin content of woody biomass, especially that originating from softwood species, increases its resistance to microbial and enzymatic action\(^{31}\). To directly expose the lignocellulose complex, plant tissues need to be micronized below the cell wall size, which is approximately 1–2 \(\mu\)m in diameter\(^{4,11}\). The digestibility of biomass cellulose was reported to be significantly enhanced on a 10 nm to 1 \(\mu\)m scale\(^{34}\). However, a recent review of physical comminution approaches has described the difficulties in achieving this condition, even with the most effective method of ball milling for 78 h, which yields an average particle size of 18.8 \(\mu\)m\(^{31}\).

2 Simultaneous enzymatic saccharification and comminution (SESC) of plants—a novel extraction method that yields non-deteriorated lignocellulosic biomass

2.1 Setup of SESC

Recently, special wet-milling equipment that achieves particle size reduction beyond the above scale was developed\(^{35}\). Ultrafine inorganic beads were used to produce nanometer-sized particles. In principle, this could allow direct access to plant cell wall components under extremely mild conditions, without using strong or toxic chemicals. We initially tested the wet ultrafine bead milling apparatus on a cedar softwood. The experimental lab scale setup, which employs zirconia beads (0.5 mm \(\phi\)), is described in Fig. 1. A mixture of jet-milled cedar powder and phosphate buffer (pH = 5.0) was comminuted at ambient temperature. During preliminary runs, we encountered problems associated with high pressure due to the generation of large particles with sizes of several \(\mu\)m. This can be attributed to entanglement of polysaccharide chains that are exposed on the particle surface during grinding. To overcome this problem, we performed simultaneous enzymatic saccharification\(^{36}\) and comminution (SESC) of the plant material. The aim was to immediately digest any exposed polysaccharides during milling.

Our basic procedure\(^{37}\) for lab scale SESC involved the following simple steps. A mixture of cedar powder and commercial enzyme (cedar powder/enzyme = 5/1 (wt/w)) in phosphate buffer (pH = 5.0) was milled for up to 2 h, while maintaining the temperature and pressure below 50°C and 1 psi, respectively. The treatment does not require heat as the bead fraction during milling increases the temperature naturally (i.e., the vessel in a milling apparatus is cooled by circulating water). The resulting slurry was centrifuged and the supernatant was collected. The precipitate was milled again under the same conditions. When required, further saccharification was allowed to proceed at 50°C for 48 h, after which the final slurry was centrifuged. The supernatants containing sugar products were pooled, while the precipitate, which consisted mainly of lignin, was washed with pure water.

2.2 Characteristics of lignocellulosic biomass extracted by SESC

Analysis confirmed that during SESC, both the viscosity of the slurry and the particle size of solids decreased dramatically over time. The final dispersed particles had an average diameter of 38 nm (Fig. 2). For comparison, the particle size obtained without enzymatic saccharification was polydisperse, ranging from several hundred to a thousand nm\(^{37}\). This clearly indicates the importance of saccharification in achieving the desired nanoscale comminution of the plant sample. The average particle size of the solid product increased to 300 nm on washing, which may be due to agglomeration of the purified nanoparticulate lignin due to its high hydrophobicity. We also applied this procedure to other woody, hard biomass (e.g., white birch and yellow pine) and herbaceous, soft biomass (e.g., a rice straw) with similar results.

Additional evaluation of the extracted compounds with respect to biofuel or food/liquor production as well as the synthesis of other functional materials yielded equally interesting data. For the former, the saccharification ratio...
Utilization of Lignocellulosic Biomass via Sustainable Process

J. Oleo Sci. 67, (9) 1059-1070 (2018)

from ultrafine wet milling was around 35% and 69% during the first and second milling, respectively. The sugar yield after two milling stages was comparable to chemical pretreatments with acid and steam explosion for softwoods\(^3\). We recently achieved a higher sugar yield with SESD by refining the extraction procedures\(^4\)\(\text{in submission}\). During high temperature dilute acid\(^5\) pretreatment of spruce for instance, saccharification of around 40% was reported using a single stage treatment, while two-stage acid hydrolysis with steam increased the yield to around 80%. However, steam explosion with concentrated acid\(\(2.5\%-4\% \text{SO}_2\)\) generated around 59%–75% sugar recovery from softwood depending on the temperature and treatment time. These comparisons emphasize the effectiveness of our method in lignocellulosic separation even at the relatively mild temperature and pH conditions employed. Subsequent in-situ fermentation of the pooled sugar-containing supernatant using a typical "sake" yeast inoculum generated ethanol\((\text{Fig. 3})\) yielded a conversion ratio approaching the maximum theoretical value. Such a high sugar to alcohol conversion was possible due to the absence of fermentation inhibitors such as furfural and phenolic derivatives\(\text{as confirmed by the absence of peaks in HPLC analysis)that are common in traditional heat and acid-based digestion of wood}^{38,39}\).

For the solid residue, the Fourier transform infrared and 'H-nuclear magnetic resonance spectra indicated a similar profile\(^3\) to that of acetylated milled-wood lignin reported previously\(^40\)–\(^42\). Moreover, the extraction ratio of aromatic compounds from the dried lignin powder by nitrobenzene oxidation\(^43\)\((27.5\%)\) was almost the same as that from neat cedar powder\((27.0\%); \text{Fig. 4}\). Conversely, lignin purified by kraft cooking\((13.2\%)\), sulfite cooking\((16.4\%)\), and alkaline cooking\((8.0\%)\) exhibited much lower extraction ratios due to the condensation of aromatic groups under strongly acidic/basic conditions. Like the lignin extract in our sample, these were highly pure and non-deteriorated. The obtained lignin film and powder also exhibited interesting properties. The lignin film was transparent with a pale brown color\(\text{(Fig. 5)}\) that was significantly different from that of the black lignin extracted via chemical processes such as kraft\(^44\), organosolv pulping\(^45\), \(\gamma\)-valerolactone-based processes\(^46\), and alkaline cooking\(^45\). The powdered lignin could be mixed with an ordinary polymer by simply heating. Kneading and hot-pressing of the lignin-polymer mixture yielded self-standing composite films. In particular, a mixture of lignin powder and stereo-random polypropylene\(\text{(L-MODU)formed self-standing films with a brown color even at a ratio of 80 w/v\% of lignin. Tensile stress–strain tests showed a significantly higher fracture strain for this film (3.7 mm/mm elongation relative to the original size; \text{Fig. 6}) compared to existing lig-
nin-polymer composites even at such a high lignin content.\textsuperscript{46, 47} The Young’s modulus of lignin-L-MODU composite films (57.6 MPa) was much higher than that of neat L-MODU films (18.4 MPa), suggesting that lignin powder can function as an antiplasticizer in polymer films. Furthermore, both unmodified and composite lignin films formed a non-flammable char residue that was several tens w/v\% of the initial weight after heating at 500°C in N\(_2\) atmosphere.\textsuperscript{198} Both the oxygen-rich lignin structure and the existence of aromatic groups may explain its incomplete combustion in N\(_2\) atmosphere, leaving a significant amount of char. This indicates that the fire-resistance of our nanoparticulate lignin, which can be converted to thermoplastic elastomers, is useful in a variety of applications. Finally, we would like to emphasize that our developed extraction method, SESC, is the first to allow use of almost the entire lignin component of the plant biomass in its natural form, that is, without chemical modification. In previous studies, chemical treatments such as hydroxypropylation,\textsuperscript{48, 49} acylation,\textsuperscript{49} and depolymerization,\textsuperscript{56, 51} were required to transform lignin plant extracts into products with high environmental and commercial value. Our method, SESC, produces a transparent lignin film, which is solvent- and fire-resistant even without chemical treatment.

Thus, SESC offers a promising approach to lignocellulosic woody biomass pretreatment for producing biofuel and other high-value materials. The present approach generates nanoscale particles, exposing the lignocellulosic biomass in plant cell walls. This allows enzymes to directly access and convert 69\% or more of the polysaccharides to soluble sugars while efficiently separating the micronized lignin. As no chemical inhibitors were formed during the non-toxic reaction process, alcohol formation from the generated sugars is achieved with a maximum theoretical yield. Furthermore, non-deteriorated high purity lignin can be recovered as a valuable material that exhibits unique properties such as flame/solvent-resistance. Current efforts aim to optimize processes to further improve sugar recovery and processing efficiency. Our basic milling process is currently being modified to achieve much higher sugar and alcohol concentrations by minimizing as well as recycling water during successive milling stages. Additionally, economic evaluations are being undertaken on a pilot scale, based on energy and enzyme costs and the extent to which these can be compensated for by the absence of toxic chemicals, which simplifies downstream processing and minimizes waste concerns. The almost complete recovery of other non-deteriorated biomass components, which positively impacts the generation of high-value secondary products, could also contribute significantly to its economic viability. In the near future, we will be able to present a pilot-scale SESC system that reduces energy costs for bead milling and exhibits ready availability of hardware for commercialization of the SESC system (in submission).

### 2.3 Unique function of lignin extracted by SESC as an excellent heatproof filler

In this section, we introduce the superior ability of non-deteriorated lignin extracted by SESC, relative to that of conventional petroleum-based materials, to function as a heatproof filler for synthetic polymers.\textsuperscript{53} As an example, we demonstrate how non-deteriorated lignin enhances the heat tolerance of poly(ethylene carbonate) (PEC). The 5\% decomposition temperature \(T_{\text{d}}\) of PEC increased from 198°C to 265°C when only 5 w/w\% of non-deteriorated lignin was added (Fig. 7). The heatproofing effect of non-deteriorated lignin on PEC is extremely one relative to other additive to carbonate-type polymer for protection against thermal degradation, e.g., citric acid.\textsuperscript{54} The heatproof properties of non-deteriorated lignin appear to be superior to those reported previously for a lignin-based heatproof filler, for which only a small increase in decomposition temperature was achieved even when several tens w/w\% lignin derivative was added.\textsuperscript{44–57} Furthermore, lignin-based heatproof fillers previously required modification with metal,\textsuperscript{54, 55} phosphorus, and/or nitrogen.\textsuperscript{56, 57} This non-deteriorated lignin did not require addition of supporting inorganic, halogen, or phosphorus compounds. The non-deteriorated lignin also acts as an antiplasticizer in PEC film as with the stereorandom polypropylene shown in Fig. 6,\textsuperscript{57} i.e., adding non-deteriorated lignin endows the PEC film with not only heat tolerance but also mechanical strength.

The \(T_{\text{d}}\) of the composite approached that of neat non-deteriorated lignin as the ratio of non-deteriorated lignin in the composite increased (Fig. 8). Furthermore, FT-IR spectroscopy revealed no hydrogen bonding between the PEC and lignin.\textsuperscript{52} These results indicate that PEC and non-
deteriorated lignin behave as individual polymers without strong molecular interactions in the composite films. The long-term heatproof properties of PEC were also improved by adding a small amount of non-deteriorated lignin. The degradation ratio of PEC at 180°C dramatically decreased with the addition of non-deteriorated lignin; that is, a composite film containing 10 w/w% non-deteriorated lignin showed 12 w/w% weight loss after 12 h of holding at 180°C, whereas neat PEC showed 95 w/w% degradation after 0.25 h of holding at 180°C.

The excellent heatproof properties of non-deteriorated lignin probably originate from its antioxidant activity; the scavenging of thermal radicals by the guaiacol groups of lignin prevents thermal degradation of the PEC main chain. Furthermore, synchrotron X-ray scattering data indicated that non-deteriorated lignin forms a platelet-shaped nanoparticle (Fig. 9). As shown in the transmission electron microscopy (TEM) image in Fig. 9, the non-deteriorated lignin exists as amorphous particles with diameters of several hundred nm. Thus, it is speculated that non-deteriorated lignin enhances the heat resistance of PEC by scavenging thermal free radicals via the guaiacol groups present on the large specific surface area of its nanoparticle platelets.

Thus, adding a small amount of non-deteriorated lignin extracted by SESC (as little as 5 w/v%) induced a remarkable increase in the decomposition temperature and thermal stability of PEC. Recently, PEC was proposed as a novel candidate for polymer Li salt electrolytes because of its unique ion conductivity. Therefore, enhancing the heat tolerance of PEC is important for its utilization at high temperatures. The heat tolerance of PEC was enhanced by non-deteriorated lignin via radical scavenging by the guaiacol groups on the platelet-shaped surface of the lignin nanoparticles. The heatproofing strategy presented here requires no toxic reagents such as halogen or phosphorus compounds. The non-deteriorated lignin can be prepared via an environmentally friendly process, i.e., SESC of plants. Therefore, the excellent heatproof properties of non-deteriorated lignin could not only positively impact the utilization of plant biomass as a high-value industrial material, but also reduce the environmental load caused by heatproof materials derived from toxic petroleum-based resources.

3 Utilization of lignin as a functional monomer

3.1 2-pyrone-4,6-dicarboxylic acid—a functional monomer obtained by metabolic degradation of lignin

In this section, we introduce another example of the novel utilization of lignin as a functional monomer. In nature, soil microorganisms degrade aromatic substances from lignin via complex metabolic pathways. Sphingomonas paucimobilis SYK-6, which was isolated from pulping waste liquor, grows on various dimeric lignin compounds. Unique degradation enzymes for low-molecular-weight compounds of lignin in SYK-6 would be suitable tools for converting lignin to useful intermediate metabolites. Via metabolic pathways of lignin employed by SYK-6, various lignin derivatives are converted within SYK-6 cells to compounds such as vanillin, vanillate, and syringate. After conversion to the metabolic intermediate 2-pyrone-4,6-dicarboxylic acid (PDC; Fig. 10) via the protocatechuate 4,5-cleavage pathway, they are completely degraded. Therefore, in SYK-6 cells, lignin derivatives are transformed into PDC and subsequently degraded. To utilize PDC as a substance with a unique structural nature containing a pyran ring with two carboxylic acid groups, the production of PDC using metabolic engineering technology was established. The application of PDC in material fields will encourage the use of lignin.

PDC, which cannot be obtained synthetically, is a chemically stable and expected to be a versatile functional substance because the pyrone ring has some useful functions, such as acceptor properties in donor–acceptor type chromophores as well as biodegradability induced by PDC hydrolase. Additionally, two carboxylic acid groups attached to the pseudoaromatic ring can be chemically modified in larger molecular systems. Crude PDC produced by the metabolic pathway was isolated by adding NaCl, before acidifying the solution. The PDC was highly soluble in polar solvents, such as water, acetone, methanol, tetrahydrofuran, and acetonitrile, but hardly soluble in nonpolar solvents, such as benzene, n-hexane, and heptane. Complexation with Na⁺ ions decreased the solubility even in polar solvents. For example, the solubility in water decreased from 182 mM (i.e., PDC) to 9.5 mM (i.e., PDC-Na⁺ complex) upon complexation. Although PDC interacts strongly with Na⁺ ions, the complex cation peak could not be detected by MALDI-TOF-MS measurements in negative ionization mode, although the molecular ion peak was observed in negative ionization mode. This is consistent with the fact that the pyrone ring has electron-accepting characteristics, especially when electron-withdrawing substituents are attached. Thus, PDC has several properties that promote its material application.

3.2 Utilization of PDC as functional monomer

The unique structure of PDC gives its ion-trapping characteristics due to multiple coordination of carboxylic, carbonyl, and ether ring oxygen atoms. PDC-Na⁺ complexes form a one-dimensional polymeric structure via Na⁺ ion coordination to the carbonyl moiety of the pyrone ring and short intermolecular hydrogen bonding of the carboxylate moieties with an O···H···O distance of 0.2452 nm. This rare and extremely short hydrogen bonding was observed in the crystal structures of electron-deficient carboxylic acidifying the solution. The PDC was highly soluble in polar solvents, such as water, acetone, methanol, tetrahydrofuran, and acetonitrile, but hardly soluble in nonpolar solvents, such as benzene, n-hexane, and heptane. Complexation with Na⁺ ions decreased the solubility even in polar solvents. For example, the solubility in water decreased from 182 mM (i.e., PDC) to 9.5 mM (i.e., PDC-Na⁺ complex) upon complexation. Although PDC interacts strongly with Na⁺ ions, the complex cation peak could not be detected by MALDI-TOF-MS measurements in negative ionization mode, although the molecular ion peak was observed in negative ionization mode. This is consistent with the fact that the pyrone ring has electron-accepting characteristics, especially when electron-withdrawing substituents are attached. Thus, PDC has several properties that promote its material application.

3.2 Utilization of PDC as functional monomer

The unique structure of PDC gives its ion-trapping characteristics due to multiple coordination of carboxylic, carbonyl, and ether ring oxygen atoms. PDC-Na⁺ complexes form a one-dimensional polymeric structure via Na⁺ ion coordination to the carbonyl moiety of the pyrone ring and short intermolecular hydrogen bonding of the carboxylate moieties with an O···H···O distance of 0.2452 nm. This rare and extremely short hydrogen bonding was observed in the crystal structures of electron-deficient carboxylic acidifying the solution. The PDC was highly soluble in polar solvents, such as water, acetone, methanol, tetrahydrofuran, and acetonitrile, but hardly soluble in nonpolar solvents, such as benzene, n-hexane, and heptane. Complexation with Na⁺ ions decreased the solubility even in polar solvents. For example, the solubility in water decreased from 182 mM (i.e., PDC) to 9.5 mM (i.e., PDC-Na⁺ complex) upon complexation. Although PDC interacts strongly with Na⁺ ions, the complex cation peak could not be detected by MALDI-TOF-MS measurements in negative ionization mode, although the molecular ion peak was observed in negative ionization mode. This is consistent with the fact that the pyrone ring has electron-accepting characteristics, especially when electron-withdrawing substituents are attached. Thus, PDC has several properties that promote its material application.

3.2 Utilization of PDC as functional monomer

The unique structure of PDC gives its ion-trapping characteristics due to multiple coordination of carboxylic, carbonyl, and ether ring oxygen atoms. PDC-Na⁺ complexes form a one-dimensional polymeric structure via Na⁺ ion coordination to the carbonyl moiety of the pyrone ring and short intermolecular hydrogen bonding of the carboxylate moieties with an O···H···O distance of 0.2452 nm. This rare and extremely short hydrogen bonding was observed in the crystal structures of electron-deficient carboxylic acidifying the solution. The PDC was highly soluble in polar solvents, such as water, acetone, methanol, tetrahydrofuran, and acetonitrile, but hardly soluble in nonpolar solvents, such as benzene, n-hexane, and heptane. Complexation with Na⁺ ions decreased the solubility even in polar solvents. For example, the solubility in water decreased from 182 mM (i.e., PDC) to 9.5 mM (i.e., PDC-Na⁺ complex) upon complexation. Although PDC interacts strongly with Na⁺ ions, the complex cation peak could not be detected by MALDI-TOF-MS measurements in negative ionization mode, although the molecular ion peak was observed in negative ionization mode. This is consistent with the fact that the pyrone ring has electron-accepting characteristics, especially when electron-withdrawing substituents are attached. Thus, PDC has several properties that promote its material application.
acids; however, to the best of our knowledge, this is the first report on biomass-derived products.

PDC also forms complexes when mixed with silver nitrate solution. In a complex of PDC and Ag⁺ ions, Ag ions strongly bind the carboxyl and carboxylate groups to form an extremely rare structure with an Ag-Ag binary core similar to that in a binuclear Ag complex of 4-fluorobenzoic acid. As shown in Fig. 11, the chelating coordination of two–COO⁻ ions could be distinguished from the side direc-

Fig. 5 A portion of dried plant biomass film consisting of nanoparticulate lignin with a diameter of 300 nm. Reproduced with permission from [37]. Copyright 2016, The Royal Society of Chemistry.

Fig. 6 Magnified typical tensile stress–strain curves of a film consisting of lignin and L-MODU at 80 w/v% of lignin (red curve), and L-MODU itself (black curve). The kneading temperature of the mixture of lignin and L-MODU is 130°C. Reproduced with permission from [37]. Copyright 2016, The Royal Society of Chemistry.

Fig. 7 Thermogravimetric analysis of the composite films and neat polymers. Reproduced with permission from [52]. Copyright 2018, The Royal Society of Chemistry.

Fig. 8 T_d of the composite films at different lignin feed ratios. Reproduced with permission from [52]. Copyright 2018, The Royal Society of Chemistry.

Fig. 9 Photograph (upper left), transmission electron microscopy (TEM) image (upper middle), distribution of functional groups on lignin (upper right), and schematic illustration of radical scavenging by guaiacol groups of non-deteriorated lignin in the composites (lower part). In the TEM image, the non-deteriorated lignin in PEC was confirmed as black objects because of preferential staining with OsO₄ of numerous carbon double bonds in precursors of lignin. Reproduced with permission from [52]. Copyright 2018, The Royal Society of Chemistry.
tions of the Ag-Ag core, while two carbonyl oxygen atoms of –COOH groups were attached to the axis of the Ag-Ag core. The PDC-Ag complex shows stronger antibacterial activity. Due to the concerted antibacterial activity of PDC and Ag ions, the minimum inhibitory concentration (MIC) of the complex is 0.025 or less, corresponding to a 10–100 fold greater activity compared to simple Ag ions.

Also, while the storage lifetime of common silver salts under ambient circumstances is short, the PDC-Ag complex shows excellent stability and can be stored for a long time.

**Fig. 10** Chemical structure of PDC.

**Fig. 11** Structure of PDC-Ag complex.

**Fig. 12** Photograph of aqueous solutions containing 7 wt% of PDC and 1 wt% of MtCl (Mt = Li, Na, K, Rb, and Cs). Reproduced with permission from [71]. Copyright 2018, Elsevier.

**Fig. 13** Photograph of glass plates joined by the PDC-based polyester and fractured after tensile testing. Reproduced with permission from [73]. Copyright 2009, Nature Publishing Group.

**Fig. 14** (Upper) Synthetic scheme of PDC thermoplastic polyesters, (middle) photographs of PDC thermoplastic polyester film, and (lower) typical tensile stress–strain curve of PDC thermoplastic polyester film. Reproduced with permission from [80]. Copyright 2013, The Society of Fiber Science and Technology, Japan.
due to discoloration by photoreduction and subsequent basic silver oxide formation, no apparent changes were found in the PDC-Ag⁺ complex. Such excellent longevity, which is presumably endowed by the unique complex structure, is also advantageous. The PDC-Ag⁺ complex exhibits a low MIC (0.025 or less) even with antibiotic-resistant bacteria such as \textit{E. coli} O157:H7, which encourages the use of PDC as a disinfection chemical from natural plant biomass resources for dairy products.

The complexation of PDC with cesium ions in preference to other alkali metal ions (i.e., Li, Na, K, and Rb ions) was also reported (Fig. 12\textsuperscript{70, 71}). Adding PDC to an aqueous mixture of CsCl and NaCl induced preferential sedimentation of Cs ions. Especially, even with a 10:1 ratio of Na ions to Cs ions (mol/mol), 88.9% of the Cs ions in the mixture were extracted by adding PDC without co-sedimentation of Na ions. Here the high coordination number of alkali ions and significant deprotonation of OH groups in the complex of PDC and Cs relative to that in the complex of PDC and Na induce three-dimensional spatially continuous binding that realizes preferential complexation between PDC and Cs. The lower electronegativity of Cs (0.86 relative to Na (1.01)) might induce significant deprotonation of OH groups in PDC and a high coordination number for metal ions. This preferential complexation between PDC and Cs presented here represents effective utilization of lignin, a plant biomass resource, as an environmentally friendly decontaminating agent for radioactive Cs in contaminated water (e.g., seawater) at Fukushima Nuclear Plant with clear ion selectivity. Thus, the complexation ability of PDC with various metal ions makes it potentially useful in diverse material sciences, e.g., medical and environmental fields.

3.3 Utilization of PDC as building block of functional polymers

Due to the presence of two carboxylic acid groups in PDC, under appropriate conditions, PDC undergoes condensation reactions with compounds having functional groups. For example, condensation polymerization of PDC at about 200°C with various diols can be promoted by a catalytic amount of \( \text{Sb}_2\text{O}_3 \textsuperscript{72} \). The Young’s moduli of PDC-based polyesters containing bis (2-hydroxyethyl) terephthalate (BHT) are comparable with that of amorphous poly (ethylene terephthalate) (PET). Additionally, the degradability of PDC-based polyesters could be controlled by changing the content of the PDC unit (i.e., pyrone ring), because both lactone ring cleavage and ester hydrolysis are considered as the major events in the main-chain scission.

The PDC-based polyester containing BHT adheres strongly to metal and glass surfaces because of chemical bonds created at the interface of the polymer layer and the metal/glass surface by a ring-opening addition reaction as shown in Fig. 13\textsuperscript{73}). These results show that the novel epoxy adhesive containing diglycidyl PDC exhibits a higher tensile strength than that of common petroleum-based epoxy adhesives such as the diglycidyl ester of bisphenol-A\textsuperscript{74}. Conversion of the carboxylic groups in PDC into cholesterol groups gives a PDC-based organogelator\textsuperscript{75, 76}.

The copolymerization of PDC, 2,4-butenediols, and succinic anhydride in the presence of a catalyst such as \( \text{Sb}_2\text{O}_3 \), \( \text{TiO} (\text{acac})_2 \) or \( \text{CH}_3\text{SO}_3\text{H} \) yields fusible, elastic, and biodegradable polyesters\textsuperscript{77}. As single component films of this polyester were brittle, blended films with poly(L-lactic acid) (PLLA) were prepared by solution casting. Tensile testing showed that these were more rigid and elastic than PLLA film, and that the maximum stress at the break point was comparable with that of the PET film. The PDC-based polyesters showed high biodegradability and rapid accelerated base hydrolysis. In particular, the biodegradability dramatically increased after copolymerization with a minute amount of PDC. Conversion of the carboxylic groups in PDC to derivatives also makes PDC a potential raw material for novel bio-based polymers such as polyamide, polyester, and polyurethane\textsuperscript{81}. An example is the transformation of carboxylic groups to ethynyl terminals for copper (1)-catalyzed azide-alkyne click reactions\textsuperscript{78, 79}. This reaction gives extremely high-molecular-weight PDC polymers with well-defined chemical structures and adhesive properties. Furthermore, polymerization of PDC via transformation of its carboxylic groups to acid chloride was also achieved, realizing efficient synthesis of thermoplastic PDC polyesters and aromatic diols with high thermal stability (Fig. 14\textsuperscript{80}). Thus, PDC, a lignin-derived monomer, can be used as a component of linear/network polymers. All these results offer design principles for biomass-based functional materials.

4 Conclusion remarks

This review describes our recent studies on the utilization of lignocellulosic biomass, especially lignin, via sustainable processes such as physico-biological extraction of plant biomass and conversion of lignin to useful compounds via biological reactions. The experimental knowledge from these studies will realize the material application of lignocellulosic biomass, creating a novel industrial field such as “Agricultural Industry”.

ACKNOWLEDGMENTS

We thank for Professor Emeritus Kiyotaka Shigehara (Tokyo University of Agriculture and Technology), and all collaborators for their kind assistance with the experimental setup. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.
COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

References
1) Kam, B.; Gruber, P.R.; Kam, M. Biorefineries-Industrial Processes and Products. Wiley-VCH, Weinheim, Germany (2006).
2) Ragauskas, A.J.; Williams, C.K.; Davison, B.H.; Britovsek, G.; Cairney, J.; Eckert, C.A.; Frederick, W.J.Jr.; Hallett, J.P.; Leak, D.J.; Liotta, C.L.; Mienzen, J.R.; Murphy, R.; Templar, R.; Tschaaplinski, T. The path forward for biofuels and biomaterials. Science 311, 484-489 (2006).
3) Vink, E.T.H.; Rabago, K.R.; Glassner, D.A.; Gruber, P.R. Applications of life cycle assessment to NatureWorksTM polylactide (PLA) production. Polym. Degrad. Stab. 80, 403-419 (2003).
4) Nishio, Y. Material functionalization of cellulose and related polysaccharides via diverse microcompositions. Adv. Polym. Sci. 205, 97-151 (2006).
5) Steinbüchel, A.; Doi, Y.; Hofrichter, M. Biopolymers. 3rd ed. Wiley-VCH, Weinheim, Germany (2002).
6) Kam, B. Production of platform chemicals and synthesis gas from biomass. Angew. Chem. Int. Ed. 46, 5056-5058 (2007).
7) Chheda, J.N.; Huber, G.W.; Dumesic, J.A. Liquid-phase catalytic processing of biomass-derived oxygenated hydrocarbons to fuels and chemicals. Angew. Chem. Int. Ed. 46, 7164-7183 (2007).
8) Kaneko, T.; Thi, T.H.; Shi, D.J.; Akashi, M. Environmentally degradable, high-performance thermoplastics from phenolic phytomonomers. Nat. Mater. 5, 966-970 (2006).
9) Corma, A.; Iborra, S.; Velty, A. Chemical routes for the transformation of biomass into chemicals. Chem. Rev. 107, 2411-2502 (2007).
10) Kemm, D.; Heublein, B.; Fink, H.P.; Bohn, A. Cellulose: fascinating biopolymer and sustainable raw material. Angew. Chem. Int. Ed. 44, 3358-3393 (2005).
11) Boething, S.R.; Sommer, E.; DiFiore, D. Designing small molecules for biodegradability. Chem. Rev. 107, 2207-2227 (2007).
12) Roman-Leshkov, Y.; Barrett, C.J.; Liu, Z.Y.; Dumesic, J.A. Production of dimethylfuran for liquid fuels from biomass-derived carbohydrates. Nature 447, 982-985 (2007).
13) Isogai, A.; Saito, T.; Fukuzumi, H. TEMPO-oxidized cellulose nanofibers. Nanoscale 3, 71-85 (2011).
14) Sarkanen, K.V.; Ludwig, C.H. Lignin: Occurrence, Formation, Structure and Reactions. (Sarkanen, K.V.; Ludwig, C.H. ed.) Wiley Interscience, New York (1971).
15) Kai, D.; Tan, M.J.; Chee, P.L.; Chua, Y.K.; Yip, Y.L.; Loh, X.J. Towards lignin-based functional materials in a sustainable world. Green Chem. 18, 1175-1200 (2016).
16) Funaka, M. A new type of phenolic lignin-based network polymer with the structure-variable function composed of 1,1-diarylp propane units. Polym. Int. 47, 277-290 (1998).
17) Takahashi, K.; Ishii, R.; Nakamura, T.; Suzuki, A.; Ebina, T.; Yoshida, M.; Kubota, M.; Nge, T.T.; Yamada, T. Flexible electronic substrate film fabricated using natural clay and wood components with cross-linking polymer. Adv. Mater. 29, 1606512 (2017).
18) Schubert, C.S. Can biofuels finally take center stage? Nature Biotechnol. 24, 777-784 (2006).
19) Aziz, S.; Sarkanen, K.V. Organosolv pulping—a review. Tappi J. 72, 169-175 (1989).
20) Luterbacher, J.S.; Rand, J.M.; Alonso, D.M.; Han, J.; Youngquist, J.T.; Maravelias, C.T.; Pfleger, B.F.; Dumesic, J.A. Nonenzymatic sugar production from biomass using biomass-derived γ-valerolactone. Science 343, 277-280 (2014).
21) Sanderson, K. Lignocellulose: A chewy problem. Nat. 474, S12-S14 (2011).
22) Chapple, C.; Ladisch, M.; Melian, R. Loosening lignin’s grip on biofuel production. Nature Biotechnol. 25, 746-748 (2007).
23) Peplow, M. Cellulosic ethanol fights for life. Nature 507, 152 (2014).
24) Alvey, C.; Reyes, A.; Sosa, F.M.; Diez, B. Enzymatic hydrolysis of biomass from wood. Microb. Biotechnol. 9, 149-156 (2016).
25) Zhang, W.; Barone, J.R.; Renneckar, S. Biomass fractionation after denaturing cell walls by glycerol thermal processing. ACS Sustainable Chem. Eng. 3, 413-420 (2015).
26) Zhang, W.; Sathitsuksanoh, N.; Barone, J.R.; Renneckar, S. Enhanced enzymatic saccharification of pretreated biomass using glycerol thermal processing (GTP). Bioresour. Technol. 199, 148-154 (2016).
27) Zhang, W.; Sathitsuksanoh, N.; Simmons, B.A.; Frazier, C.E.; Barone, J.R.; Renneckar, S. Revealing the thermal sensitivity of lignin during glycerol thermal processing through structural analysis. RSC Adv. 6, 30234-30246 (2016).
28) Zhu, J.Y.; Pan, X.J.; Wang, G.S.; Gleisner, R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. Biores. Technol. 100, 2411-2418 (2009).
29) Björkman, A. Isolation of lignin from finely divided wood with neutral solvents. Nature 174, 1057-1058 (1954).
30) Ralph, J.; MacKay, J.J.; Hatfield, R.D.; O’Malley, D.M.; Whetten, R.W.; Sederoff, R.R. Abnormal lignin in a loblolly pine mutant. Science 277, 235-239 (1997).
31) Zhu, J.Y.; Pan, X.J. Woody biomass pretreatment for cellulolic ethanol production: technology and energy consumption evaluation. Bioresour. Technol. 100, 4992-5002 (2010).
32) Zheng, Y.; Pan, Z.; Zhang, R. Overview of biomass pretreatment for cellulolic ethanol production. Int. J. Agric. Biol. Eng. 2, 51-68 (2009).
33) Licari, A.; Monlau, F.; Solhy, A.; Buche, P.; Barakat, A. Comparison of various milling modes combined to the enzymatic hydrolysis of lignocellulosic biomass for bioenergy production: Glucose yield and energy efficiency. Energy 102, 335-342 (2016).
34) Teramoto, Y.; Tanaka, N.; Lee, S.H.; Endo, T. Pretreatment of eucalyptus wood chips for enzymatic saccharification using combined sulfuric acid-free ethanol cooking and ball milling. Biotechnol. Bioeng. 99, 75-85 (2007).
35) Tanaka, Y.; Inkyo, M.; Yumoto, R.; Nagai, J.; Takano, M.; Nagata, S. Nanoparticulation of poorly water soluble drugs using a wet-mill process and physicochemical properties of the nanopowders. Chem. Pharm. Bull. 57, 1050-1057 (2009).
36) Mais, U.; Esteghlalian, A.R.; Saddler, J.N.; Mansfield, S.D. Enhancing the enzymatic hydrolysis of lignocellulosic materials using simultaneous ball milling. Appl. Biochem. Biotech. 98-100, 815-832 (2002).
37) Shikinaka, K.; Otsuka, Y.; Navarro, R.R.; Nakamura, M.; Shimokawa, T.; Nojiri, M.; Tanigawa, R.; Shigehara, K. Simple and practicable process for lignocellulosic biomass utilization. Green Chem. 18, 5962-5966 (2016).
38) Klinke, H.B.; Thomsen, A.B.; Abring, B.K. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl. Microbiol. Biotech. 66, 10-26 (2004).
39) Almeida, J.R.; Modig, T.; Petersson, A.; Hahn-Hägerdal, B.; Lidén, G.; Gorwa-Grauslund, M.F. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by Saccharomyces cerevisiae. J. Chem. Technol. Biotechnol. 82, 340-349 (2007).
40) Lundquist, L. NMR-studies of lignins. 4. Investigation of spruce lignin by H-1-NMR spectroscopy. Acta Chem. Scand. B 34, 21-26 (1980).
41) Lundquist, K. NMR-Studies of Lignins. 5. Investigation of non-derivatized spruce and birch lignin by H-1-NMR spectroscopy. Acta Chem. Scand. B 35, 497-501 (1981).
42) Lundquist, K. Methods in Lignin Chemistry (Lin, S.Y.; Dence, C.W. eds.), Springer-Verlag, New York, NY, USA, pp. 242-249 (1992).
43) Creighton, R.H.J.; Gibbs, R.D.; Hibbert, H. Studies on lignin and related compounds. LXXV. alkaline nitro-benzene oxidation of plant materials and application to taxonomic classification. J. Am. Chem. Soc. 66, 32-37 (1944).
44) Adler, E. Lignin chemistry-past, present and future. Wood Sci. Technol. 11, 169-218 (1977).
45) Shikinaka, K.; Fujii, N.; Egashira, S.; Murakami, Y.; Nakamura, M.; Otsuka, Y.; Ohara, S.; Shigehara, K. Poly-functional nanometric particle obtained from lignin, a woody biomass resource. Green Chem. 12, 1914-1916 (2010).
46) Sivasankarapillai, G.; Li, H.; McDonald, A.G. Lignin-based triple shape memory polymers. Biomacromolecules 16, 2735-2742 (2015).
47) Li, Y.; Mlynar, J.; Sarkanes, S. The first 85% kraft lignin-based theroplastics. J. Polym. Sci. B 35, 1899-1910 (1997).
48) Wu, L.C.-F.; Glasser, W.G. Engineering plastics from lignin. I. Synthesis of hydroxypropyl lignin. J. Appl. Polym. Sci. 29, 1111-1123 (1984).
49) Li, Y.; Sarkanes, S. Miscible Blends of Kraft Lignin Derivatives with Low-$T_g$ Polymers. Macromolecules 38, 2296-2306 (2005).
50) Rahimi, A.; Ulbrichl, A.; Coon, J.J.; Stahl, S.S. Formic-acid-induced depolymerization of oxidized lignin to aromatics. Nature 515, 249-252 (2014).
51) Htet, A.N.; Noguchi, M.; Ninomiya, K.; Tsuge, Y.; Kuroda, K.; Kajita, S.; Masui, E.; Katayama, Y.; Shikinaka, K.; Otsuka, Y.; Nakamura, M.; Honda, R.; Takahashi, K. Application of microalgal hydrolysate as a fermentation medium for microbial production of 2-pyrone 4,6-dicarboxylic acid. J. Biosci. Bioeng. 125, 717-722 (2018).
52) Shikinaka, K.; Socome, H.; Kubota, Y.; Tominaga, Y.; Nakamura, M.; Navarro, R.R.; Otsuka, Y. A small amount of nanoparticulated plant biomass, lignin, enhances heat tolerance for poly(ethylene carbonate). J. Mater. Chem. A, 6, 837-839 (2018).
53) Phillips, O.; Schwartz, J.M.; Kohl, P.A. Thermal decomposition of poly(propylene carbonate): End-capping, additives, and solvent effects. Polym. Degrad. Stabil. 125, 129-139 (2016).
54) Liu, L.; Qian, M.; Song, P.; Huang, G.; Yu, Y.; Fu, S. Fabrication of green lignin-based flame retardants for enhancing the thermal and fire retardancy properties of polypropylene/wood composites. ACS Sustainable Chem. Eng. 4, 2422-2431 (2016).
55) Yu, Y.; Song, P.; Jin, C.; Fu, S.; Zhao, L.; Wu, Q.; Ye, J.
Catalytic effects of nickel (cobalt or zinc) acetates on thermal and flammability properties of polypropylene-modified lignin composites. *Ind. Eng. Chem. Res.* **51**, 12367-12374 (2012).

56) Yu, Y.; Fu, S.; Song, P.; Luo, X.; Jin, Y.; Lu, F.; Wu, Q.; Ye, J. Functionalized lignin by grafting phosphorus-nitrogen improves the thermal stability and flame retardancy of polypropylene. *Polym. Degrad. Stabil.* **97**, 541-546 (2012).

57) Ferry, L.; Dorez, G.; Taguet, A.; Otazaghine, B.; Lopez-Cuesta, J.M. Chemical modification of lignin by phosphorus molecules to improve the fire behavior of polybutylene succinate. *Polym. Degrad. Stabil.* **113**, 135-143 (2015).

58) Azadfar, M.; Gao, A.H.; Bule, M.V.; Chen, S. Structural characterization of lignin: A potential source of antioxidants guaiacol and 4-vinylguaiacol. *Int. J. Biol. Macromol.* **75**, 58-66 (2015).

59) Tominaga, Y. Ion-conductive polymer electrolytes based on poly(ethylene carbonate) and its derivatives. *Polym. J.* **49**, 291-299 (2017).

60) Masai, E.; Shinohara, S.; Hara, H.; Nishikawa, S.; Katayama, Y.; Fukuda, M. Genetic and Biochemical Characterization of a 2-Pyrone-4,6-Dicarboxylic Acid Hydrolase Involved in the Protocatechuateg-4,5-Cleavage Pathway of *Sphingomonas paucimobilis* SYK-6. *J. Bacteriol.* **181**, 55-62 (1999).

61) Otsuka, Y.; Nakamura, M.; Shigehara, K.; Sugimura, K.; Masai, E.; Ohara, S.; Katayama, Y. Efficient production of 2-pyrene-4,6-dicarboxylic acid as a novel polymer-based material from protocatechuate 4,5-Cleavage intermediate of lignin. *J. Oleo Sci.* **63**, 2000-2007 (2008).

62) Hirano, K.; Minakata, S.; Komatsu, M.; Mizoguchi, J. Intense blue luminescence of 3,4,6-triphenyl-α-pyrene in the solid state and its electronic characterization. *J. Phys. Chem. A.* **106**, 4868-4871 (2002).

63) Collings, C.; Parsons, A.C.; Porre’s, L.; Beeby, A.; Batsanov, A.S.; Howard, J.A.K.; Lydon, D.P.; Low, P.J.; Fairlamb, I.J.S.; Marder, T.B. Optical properties of donor–acceptor phenylene-ethynylene systems containing the 6-methylpyran-2-one group as an acceptor. *Chem. Commun.* 2666-2668 (2005).

64) Kersten, P.J.; Dagley, S.; Whittaker, J.W.; Arciero, D.M.; Lipscomb, J.D. 2-pyrene-4,6-dicarboxylic acid, a catabolite of gallic acids in Pseudomonas species. *J. Bacteriol.* **152**, 1154-1162 (1982).

65) Michinobu, T.; Bito, M.; Yamada, Y.; Katayama, Y.; Noguchi, K.; Masai, E.; Nakamura, M.; Ohara, S.; Shigehara, K. Molecular properties of Sphingomonas accesible PDC of lignin isolated by fractional precipitation with Na⁺ ion. *Bull. Chem. Soc. Jpn.* **80**, 2436-2442 (2007).

66) Day, V.W.; Hussain, Md.A.; Kang, S.O.; Powell, D.; Lushington, G.; Bowman-James, K. Encircled proton. *J. Am. Chem. Soc.* **129**, 8692-8693 (2007).

67) Zhu, H.; Zhang, X.; Liu, X.; Wang, X.; Liu, G.; Usman, A.; Fun, H. Clear Ag-Ag bonds in three silver(1) carboxylate complexes with high cytotoxicity properties. *Inorg. Chem. Commun.* **6**, 1113-1116 (2003).

68) Bito, M.; Michinobu, T.; Katayama, Y.; Otsuka, Y.; Nakamura, M.; Ohara, S.; Masai, E.; Shigehara, K. 2-Pyrene-4,6-dicarboxylic acid as a source of green-plastics and anti-bacterial chemicals. *Trans. Mater. Res. Soc. Jpn.* **33**, 1165-1168 (2008).

69) Kikuchi, Y. Antibacterial metals. *Materia Jpn.* **39**, 146-150 (2000).

70) Shikinaka, K.; Otsuka, Y.; Iguchi, Y.; Nakamura, M.; Itoh, Y.; Masai, E.; Katayama, Y.; Shigehara, K. Preferential cesium trapping by 2-pyrene-4,6-dicarboxylic acid (PDC) obtained from a metabolic intermediate of lignin, a woody biomass resource. *J. Nucl. Sci. Technol.* **53**, 1256-1259 (2016).

71) Bito, M.; Otsuka, Y.; Nakamura, M.; Masai, E.; Katayama, Y.; Shigehara, K.; Shikinaka, K. Unique complexation behavior of alkali metal ions and 2-pyrene-4,6-dicarboxylic acid (PDC) obtained from a metabolic intermediate of lignin. *Polym. J.* **40**, 68-75 (2008).

72) Michinobu, T.; Hisida, M.; Sato, M.; Katayama, Y.; Masai, E.; Nakamura, M.; Otsuka, Y.; Ohara, S.; Shigehara, K. Polyesters of 2-pyrene-4,6-dicarboxylic acid (PDC) obtained from a metabolic intermediate of lignin. *Polym. J.* **40**, 207-302 (2009).

73) Hasegawa, Y.; Shikinaka, K.; Katayama, Y.; Kajita, S.; Masai, E.; Nakamura, M.; Otsuka, Y.; Ohara, S.; Shigehara, K. Tenacious epoxy adhesives prepared from lignin-derivative stable metabolic intermediate. *Sen’i Gakukaishi* **75**, 359-362 (2009).

75) Michinobu, T.; Hiraki, K.; Fujii, N.; Shikinaka, K.; Katayama, Y.; Masai, E.; Nakamura, M.; Otsuka, Y.; Ohara, S.; Shigehara, K. Organogels of lignin-derived stable metabolic intermediate, 2-pyrene-4,6-dicarboxylic acid (pdc), bearing cholesteryl groups. *Chem. Lett.* **39**, 400-401 (2010).

76) Michinobu, T.; Hiraki, K.; Fujii, N.; Shikinaka, K.; Katayama, Y.; Masai, E.; Nakamura, M.; Otsuka, Y.; Ohara, S.; Shigehara, K. Liquid crystallinity and organogelation behavior of lignin-derived metabolic intermediate bearing cholesterol groups. *Bull. Chem. Soc. Jpn.* **84**, 667-674 (2011).
S.; Shigehara, K. Fusible, elastic, and biodegradable polyesters of 2-pyrone-4,6-dicarboxylic acid (PDC). Polym. J. 41, 1111-1116 (2009).
78) Michinobu, T.; Inazawa, Y.; Hiraki, K.; Katayama, Y.; Masai, E.; Nakamura, M.; Ohara, S.; Shigehara, K. A novel biomass-based polymer prepared from lignin-derived stable metabolic intermediate by copper (I)-catalyzed azide-alkyne click reaction. Chem. Lett. 37, 154-155 (2008).
79) Michinobu, T.; Hiraki, K.; Inazawa, Y.; Katayama, Y.; Masai, E.; Nakamura, M.; Ohara, S.; Shigehara, K. Click synthesis and adhesive properties of novel biomass-based polymers from lignin-derived stable metabolic intermediate. Polym. J. 43, 648-653 (2011).
80) Shikinaka, K.; Hashimoto, Y.; Kajita, S.; Masai, E.; Katayama, Y.; Nakamura, M.; Otsuka, Y.; Ohara, S.; Shigehara, K. Thermoplastic polyesters of 2-pyrone-4,6-dicarboxylic acid (PDC) obtained from a metabolic intermediate of lignin. Sen’i Gakkaishi 69, 39-47 (2013).