Unassisted solar lignin valorisation using a compartmented photo-electro-biochemical cell

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Lignin is a major component of lignocellulosic biomass. Although it is highly recalcitrant to break down, it is a very abundant natural source of valuable aromatic carbons. Thus, the effective valorisation of lignin is crucial for realising a sustainable biorefinery chain. Here, we report a compartmented photo-electro-biochemical system for unassisted, selective, and stable lignin valorisation, in which a TiO2 photocatalyst, an atomically dispersed Co-based electrocatalyst, and a biocatalyst (lignin peroxidase isozyme H8, horseradish peroxidase) are integrated, such that each system is separated using Nafion and cellulose membranes. This cell design enables lignin valorisation upon irradiation with sunlight without the need for any additional bias or sacrificial agent and allows the protection of the biocatalyst from enzyme-damaging elements, such as reactive radicals, gas bubbles, and light. The photo-electro-biochemical system is able to catalyse lignin depolymerisation with a 98.7% selectivity and polymerisation with a 73.3% yield using coniferyl alcohol, a lignin monomer.
Biomass is considered a promising replacement for fossil fuels because it is the most abundant carbon source in nature and is carbon neutral\textsuperscript{11,12}. There has been remarkable progress on biomass conversion technologies, especially the conversion of sugar or starch crops to biofuels and various chemicals. However, the use of edible biomass as a feedstock is controversial in terms of ethics and cost\textsuperscript{3}. Hence, waste biomass, so-called lignocellulosic biomass, such as wood residues, straw, and crop stover, have recently emerged as promising carbon sources\textsuperscript{2}.

Lignin is one of the three major components of lignocellulosic biomass, together with cellulose and hemicellulose. It is nature’s most abundant source of aromatic carbon compounds and can be potentially transformed into high-value products\textsuperscript{4–11}. However, because of its complex/irregular chemical structure and currently limited processing technology, more than 99% of lignin is abandoned or burned\textsuperscript{12}. To make lignocellulosic biomass a more compatible renewable carbon source, an effective method for lignin valorisation is vital. The key issue here is finding effective strategies for the selective cleavage of carbon–oxygen bonds (C–O–C), more specifically, the β-ether (β-O-4) bond), which connects the three basic aromatic units (syringyl, coniferyl alcohol, and sinapyl alcohol); this is a critical step for the depolymerisation of lignin to yield valuable aromatic chemicals and feedstocks\textsuperscript{13–17}.

Various treatment technologies including physical (ball milling, ultrasonication, plasma irradiation, and microwave heating), chemical (using an organic or inorganic acid), or catalytic (with a heterogeneous catalyst) methods have been used for lignin valorisation\textsuperscript{18–21}. However, these processes are usually energy-intensive, requiring high temperatures and pressures, and environmentally unfriendly, producing chemical waste. Most importantly, selective carbon–oxygen bond cleavage is not plausible using these conventional methods. However, microorganisms including fungi and bacteria have been selectively degrading lignin for more than 300 million years, although the detailed mechanism remains elusive\textsuperscript{22}. In particular, the lignin peroxidase isozyme H8 (LIPH8) biocatalyst from white rot fungi has received much attention because of its exceptional ability for the selective cleavage of β-O-4 bonds in lignin\textsuperscript{23–25}. However, a critical limitation of biocatalyst systems is that hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), which acts as an electron acceptor, must be provided from an external source, and its high concentration is detrimental to enzyme stability, limiting the scale-up of these systems.

The integration of the H\textsubscript{2}O\textsubscript{2} generation system with the biocatalytic conversion system allows for continuous production and utilisation of H\textsubscript{2}O\textsubscript{2}, which would allow the use of low concentrations of H\textsubscript{2}O\textsubscript{2} and the production of large-scale devices. The current method of producing H\textsubscript{2}O\textsubscript{2} is predominantly based on the anthraquinone process, which consists of multi-step processes and requires additional separation steps in the presence of high pressure H\textsubscript{2} and expensive precious metal catalysts\textsuperscript{26}. Direct reaction of H\textsubscript{2} and O\textsubscript{2} on a catalyst is a simple and clean process, but this requires high-pressure gases (H\textsubscript{2} and O\textsubscript{2})\textsuperscript{27,28}. Therefore, the anthraquinone process and direct H\textsubscript{2}O\textsubscript{2} synthesis are not controversial in terms of ethics and cost\textsuperscript{3}. Hence, waste biomass, together with cellulose and hemicellulose. It is nature’s most abundant source of aromatic carbon compounds and can be potentially transformed into high-value products\textsuperscript{4–11}. However, because of its complex/irregular chemical structure and currently limited processing technology, more than 99% of lignin is abandoned or burned\textsuperscript{12}. To make lignocellulosic biomass a more compatible renewable carbon source, an effective method for lignin valorisation is vital. The key issue here is finding effective strategies for the selective cleavage of carbon–oxygen bonds (C–O–C), more specifically, the β-ether (β-O-4) bond), which connects the three basic aromatic units (syringyl, coniferyl alcohol, and sinapyl alcohol); this is a critical step for the depolymerisation of lignin to yield valuable aromatic chemicals and feedstocks\textsuperscript{13–17}.

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On the other hand, photocatalysis can also produce H\textsubscript{2}O\textsubscript{2} in a straightforward, clean manner and can be operated under ambient conditions like electrocatalytic H\textsubscript{2}O\textsubscript{2} production. On the irradiation of the photocatalyst with light, excited electrons are produced in the conduction band, and these are then utilised for O\textsubscript{2} reduction, producing H\textsubscript{2}O\textsubscript{2}. Recently, several groups have combined this photocatalytic H\textsubscript{2}O\textsubscript{2} system with biocatalysts and applied them for various chemical reactions. For example, a modified powder-type TiO\textsubscript{2} photocatalyst was utilised for H\textsubscript{2}O\textsubscript{2} generation, and it was integrated with a biocatalytic system of peroxygenases for the selective oxyfunctionalisations of carbon–hydrogen bonds, which has been one of the major challenges in organic synthesis\textsuperscript{32–35}. However, this integrated system (biocatalytic system with photocatalytically generated H\textsubscript{2}O\textsubscript{2}) has been rarely applied for lignin valorisation, and most of the research has been focused on developing an efficient powder-type photocatalyst, such as Nb\textsubscript{2}O\textsubscript{5}/TiO\textsubscript{2} heterojunction, metal-doped TiO\textsubscript{2} or graphene–TiO\textsubscript{2} nanocomposite for direct lignin conversion rather than for H\textsubscript{2}O\textsubscript{2} production\textsuperscript{12,36}. One of the important limitations of the integrated system is that, for efficient H\textsubscript{2}O\textsubscript{2} generation using powder-type photocatalysts, additional chemicals are required to scavenge the remaining hole in the valence band. Most crucially, the reactive oxygen species (ROS) generated during the photocatalytic reaction not only destabilise the biocatalyst but also cause the random cracking/breaking of the lignin structure, thus decreasing the selectivity of lignin conversion dramatically.

Here, we propose a compartmented photo-electro-biochemical cell, in which three catalytic systems (a photocatalyst for photovoltage generation, an electrocatalyst for H\textsubscript{2}O\textsubscript{2} production, and a biocatalyst for lignin valorisation) are integrated for selective lignin valorisation without the need for electrical energy or additional chemicals (Fig. 1). Importantly, the compartmentalisation of the three catalytic systems using Nafion and cellulose membranes stabilises the biocatalyst from enzyme-damaging elements, such as ROS, the high concentration of H\textsubscript{2}O\textsubscript{2}, shear stress from gas (O\textsubscript{2}) bubbles, and light. Moreover, because the lignin and photocatalyst are separated from each other, the dark-coloured native lignin does not reduce the light adsorption efficiency of the photocatalyst, a problem that has plagued the use of a photocatalyst for lignin valorisation (Fig. 1). The compartmented photo-electro-biochemical cell can catalyse the depolymerisation of lignin dimer with high selectivity (>95%) and the polymerisation of a benchmark lignin monomer, coniferyl alcohol, in 73.3% yield. The catalytic performance of the three-compartment system is superior to those of single-compartment and two-compartment catalytic systems.

**Results**

**Design of the photo-electro-biochemical system.** The designed photo-electro-biochemical system is composed of three compartments (photocatalyst anode, electrocatalyst cathode, and biocatalyst part), as shown in Fig. 1. Each cell is filled with an appropriate solution, and the cells are separated by Nafion and cellulose membranes. In the first compartment, a semiconductor photocatalyst receives the solar energy, and photoexcitation generates charge carriers. The photogenerated hole in the valence band oxidises water to O\textsubscript{2}, and the electrons move to the second compartment, where the electrocatalyst selectively reduces O\textsubscript{2} to H\textsubscript{2}O\textsubscript{2}. The produced H\textsubscript{2}O\textsubscript{2} is transferred through the size-selective cellulose membrane to the third compartment, where lignin valorisation via the biocatalyst occurs with the aid of the permeated H\textsubscript{2}O\textsubscript{2}. Importantly, the two membranes between each catalytic system prevent the permeation of O\textsubscript{2} bubbles and ROS, such as hydroxyl radicals (•OH) and superoxide anions (•O\textsuperscript{2–}), thus protecting the biocatalyst. In addition, the compartmented design ensures that the biocatalyst is not exposed to a high concentration of H\textsubscript{2}O\textsubscript{2} and intense light because of the gradual permeation of H\textsubscript{2}O\textsubscript{2} through the cellulose membrane and the
Unassisted photo-electrochemical H₂O₂ production. As a photoanode for water oxidation, a rutile TiO₂ nanowire film was used. This film was hydrothermally grown on a fluorine-doped tin oxide (FTO) glass substrate and was subsequently annealed in a hydrogen atmosphere to improve the charge transfer properties of TiO₂ (denoted H:TiO₂) [37]. The X-ray diffraction (XRD) pattern of H:TiO₂ (Supplementary Fig. 2a) shows two diffraction peaks at 36.1° and 62.8°, which is consistent with that of rutile TiO₂. There was no significant change in the UV−vis spectra of the TiO₂ films upon hydrogen treatment (Supplementary Fig. 2b). The scanning electron microscopy (SEM) image of H:TiO₂ (Supplementary Fig. 3) reveals that a homogeneous film was formed on the FTO substrate, consisting of vertically aligned nanowire arrays of 100–200 nm in diameter. Phosphate borate solution at pH 4.5 was utilised as an electrolyte. Under sunlight illumination, the photocurrent density of the bare TiO₂ nanowire photoanode at 1.23 V (vs. reversible hydrogen electrode, RHE) was 0.97 mA cm⁻², which is far higher than that of planar-type TiO₂ electrode (0.11 mA cm⁻²) because of the improved charge transfer rate and increased surface area due to the nanowire morphology (Supplementary Fig. 4a). Upon hydrogen treatment of TiO₂ (H:TiO₂), the photocurrent density was further increased to 1.25 mA cm⁻² with almost the same onset potential of 0.40 V (vs. RHE) and there was no sign of a decrease in its performance for 12 h (Fig. 2a and Supplementary Fig. 4).

The photovoltage generated at the anode is used to catalyse O₂ reduction at the cathode, completing the unassisted production of H₂O₂. As H₂O₂ production electrocatalysts, cobalt porphyrins have shown high activity and selectivity for the two-electron oxygen reduction reaction (ORR) [39,40]. However, these molecular catalysts are incompatible with photocatalysis cells because of their instability [41]. In order to endow catalytic stability while preserving the intrinsic activity of the Co-porphyrin-based molecular catalysts, we used a silica-protective-layer-assisted synthesis [42,43] (see the “Methods” section for detail). In the resulting catalyst, atomically dispersed Co–N₅ sites are homogeneously generated on carbon nanotubes (denoted Co–N/CNT) without the aggregation of the Co species (Supplementary Fig. 5a, b). For comparison, a cobalt porphyrin molecular catalyst immobilised on the CNTs was also prepared (CoTMPP/CNT). The Co–N/CNT catalyst was found to contain 0.8 and 1.3 wt% of Co and N, respectively, as determined by inductively coupled plasma optical emission spectrometry and combustion elemental analysis, respectively. Co 2p and N 1s X-ray photoelectron spectroscopy (XPS) scans (Supplementary Fig. 6) suggest the presence of oxidised Co species (satellite peaks) and four types of N species on the surface of the Co–N/CNT catalyst. Extended X-ray absorption fine structure analysis (Supplementary Fig. 7) reveals that the Co–N coordination bonds (Co–N₅ sites) of the cobalt porphyrin precursor were retained in the Co–N/CNT catalyst after the high-temperature treatment, and there is no observable Co–Co bond peak, indicating that the aggregation of Co metal was minimised by the silica-protective-layer strategy.

The ORR activity and selectivity of the Co–N/CNT and CoTMPP/CNT catalysts were investigated using the rotating ring disk electrode (RRDE) technique (Fig. 2b and Supplementary Fig. 8). The ORR polarisation curve shows that the Co–N/CNT catalyst started to generate the H₂O₂ production current from 0.78 V (vs. RHE) in phosphate borate solution at pH 4.5 (Fig. 2b). This onset potential is very close to thermodynamic equilibrium potential for two-electron ORR with an overpotential of only 0.04 V (Supplementary Note 1), indicating the excellent catalytic activity of the Co–N/CNT catalyst. In contrast, CoTMPP/CNT and pristine CNT required much larger overpotentials of 0.33 and 0.47 V (Fig. 2b), respectively, suggesting that the heat-treated Co–N₅ structure results in high activity. RRDE and
Koutecký–Levich analyses show the consistently high H₂O₂ selectivity (48–62%) of the Co–N/CNT catalyst (Supplementary Fig. 8b). The molecular CoTMPP/CNT catalyst shows a better H₂O₂ selectivity of 60–64% than Co–N/CNT, which is attributed to its well-defined Co–N₄ structure. However, after being subjected to prolonged potential cycling tests, CoTMPP/CNT showed a decline in the activity for H₂O₂ production of around 20%, whereas the Co–N/CNT catalyst mostly maintained its activity (Fig. 2b). Scanning transmission electron microscopy (STEM) images of Co–N/CNT before and after the stability test were nearly the same, indicating that locally dispersed Co species are not agglomerated or detached from CNT support (Supplementary Fig. 5). The Co–N/CNT catalyst is also active for electrochemical H₂O₂ production at low pH (0.1 M HClO₄), extending the potential applicability of this catalyst to photoelectrocatalytic H₂O₂ production, as well as use with other photoanodes and biocatalysts (Supplementary Fig. 9).

Next, a large-area cathode composed of the Co–N/CNT catalyst was prepared on a piece of carbon paper for photoelectrocatalytic H₂O₂ production. The photoelectrocatalytic activities of the photoanode and cathode for water oxidation and the ORR, respectively, were tested in phosphate borate solution using linear sweep voltammetry (LSV) measurements. The operating current of the integrated photoelectrocatalytic cell could be estimated from the intersection of the LSV curves of the H₂TiO₄ photoanode and Co–N/CNT cathode (Fig. 2c). The intersection point in the LSV of H₂TiO₄ and Co–N/CNT was 0.62 mA, which far exceeds that of H₂TiO₄ and CoTMPP/CNT (0.09 mA), as well as those of H₂TiO₄ and pristine CNT (0.07 mA), highlighting the importance of an effective electrocatalyst for unassisted H₂O₂ production (Fig. 2c and Supplementary Fig. 10).

To verify the stability of our integrated photoelectrochemical H₂O₂ production system, the amount of H₂O₂ generated on the Co–N/CNT cathode was estimated as a function of time using a colorimetric method with N,N-diethyl-p-phenylenediamine (DPD) (Fig. 2d). We observed continuous production of H₂O₂ over 6 h of reaction, and found that the H₂O₂ production rate can be easily increased by scaling up the size of electrodes and the reactor (Supplementary Fig. 11). In addition, the H₂O₂ produced at the cathode freely diffused into the biocatalyst cell through the cellulose membrane (Supplementary Fig. 12), enabling the utilisation of H₂O₂ by the biocatalyst for lignin depolymerisation and biopolymer synthesis.

**Photo-electro-biochemical lignin dimer depolymerisation.** There are many reported lignin model compounds, such as ferulic acid, vanillyl alcohol isoeugenol, and benzyl alcohol. We selected lignin dimer as a representative model compound to observe the specific cleavage of β-O-4. For the selective cleavage of the lignin dimer into 3,4-dimethoxybenzaldehyde (a derivative of vanillin), the LiPH8 biocatalyst was used, along with the photoelectrochemically generated H₂O₂ (Supplementary Fig. 13). Because our three-compartment cell design protects the biocatalyst from deactivation, the enzyme activity was maintained throughout the reaction (Fig. 3a and Supplementary Fig. 14). The depolymerisation conversion efficiency and selectivity for the lignin dimer were 93.7% and 98.7%, respectively, in the three-compartment photo-electro-biochemical system (Fig. 3b, c). A control reaction carried out in the absence of the LiPH8 enzyme in the three-compartment cell indicated low conversion and selectivity, suggesting that the biocatalyst is essential for selective lignin valorisation (Supplementary Fig. 15).
For comparison, we also tested lignin depolymerisation in single-compartment and two-compartment systems. In the single-compartment system, the powder-type TiO₂ photocatalyst, the LiPH₈ biocatalyst, and the lignin dimer were placed in the same compartment (Fig. 3d and Supplementary Fig. 1c). In this environment, lignin conversion can take place in two possible ways: direct lignin conversion by the charge carriers generated by the TiO₂ photocatalyst or biocatalytic lignin conversion using LiPH₈ with the aid of the photogenerated H₂O₂. The obtained lignin dimer conversion and selectivity were 37.3% and 34.8%, respectively (Fig. 3e, f). To investigate the role of the enzyme in the single-compartment system, we performed the lignin conversion experiment in the absence of LiPH₈. Interestingly, a similar conversion and selectivity were obtained without LiPH₈ (Supplementary Fig. 16) and very little H₂O₂ was generated in the single compartment (Supplementary Fig. 17), which indicates that the reaction mainly proceeds via direct photocatalytic lignin conversion by the TiO₂ photocatalyst. The presence of many unidentified peaks using gas chromatography–mass spectroscopy (GC–MS) analysis also revealed that many side reactions take place upon the direct photocatalytic conversion of lignin (Supplementary Figs. 18–22). In the single-compartment system, solar light first excites the photocatalyst, generating holes and electrons that initiate the photochemical reaction and generate intermediate radical species (·OH and ·O₂⁻, respectively). These intermediate radicals can directly attack the biocatalyst, leading to a significant drop in activity. As for other proteins/enzymes, such as chymotrypsin, lysozyme, ribonuclease, and formate dehydrogenase, it was found that LiPH₈ in this study also rapidly lost its activity upon irradiation and in the presence of bubbles of O₂ gas (Supplementary Fig. 14)⁴⁵–⁴⁷. In particular, ultraviolet (UV) light directly destroys the protein structures ⁴⁵. Furthermore, gas bubbling also alters the protein structure upon adsorption of the biocatalyst at gas–liquid interfaces ⁴⁶,⁴⁷. These factors (ROS,
light, and O₂ gas bubbling) are the main reasons for the low selectivity of the single-compartment system.

In the two-compartment system, the photoanode (H₂TiO₃) and electrocatalyst cathode (Co-O/N/CNT) were separated by a Nafion proton exchange membrane, but the cellulose membrane was removed between the cathode and biocatalyst parts (Fig. 3g and Supplementary Fig. 1d). This configuration prevents the direct photochemical lignin conversion observed in the single-compartment system and restrains the deactivation of LiPH₈ biocatalyst by the photogenerated holes and •OH radicals in the anodic part. Furthermore, the remaining area was blackened and the H₂TiO₃ photoanode was covered from behind, as shown in Supplementary Fig. 1e, to shield the biocatalyst from UV light and inhibit its photo-deactivation as in the three-compartment system. The two-compartment cell showed increased selectivity of over 48% (Fig. 3i), which mainly arises from biocatalytic lignin conversion with the help of the in situ photogenerated H₂O₂ (Supplementary Fig. 23), because only 3% selectivity was obtained when parallel reaction was carried out in the absence of the LiPH₈ biocatalyst (Supplementary Fig. 24). However, lignin dimer conversion was still very low (<25%) because the LiPH₈ was exposed to O₂ bubbles, which are detrimental to enzyme activity (Fig. 3h and Supplementary Fig. 14). Moreover, •O₂⁻ radical intermediate generated during the O₂ reduction also has an adverse effect on the LiPH₈ enzyme activity.

In the three-compartment photo-electro-biochemical system, we verified that the concentrations of •OH and •O₂⁻ radicals were very low in the enzyme cell compared to those in the anode and cathode cells, respectively (Supplementary Figs. 25 and 26), resulting in high conversion and selectivity of lignin dimer depolymerisation. The gradual increase of H₂O₂ concentration in the three-compartment cell is also a reason for the high performance, as this helps to maintain low concentrations of H₂O₂ in the enzyme cell. When the initial concentrations were 1 and 10 mM, the conversion efficiencies were 82% and 21%, respectively. These values are lower than that of a three-compartment system (93.7%) because of enzyme deactivation due to high H₂O₂ concentration (Supplementary Fig. 27). When we fed a small amount of H₂O₂ every 15 min, the conversion efficiency was increased, indicating that the continuous and low concentration of H₂O₂ supply is preferred for the efficient biocatalytic lignin degradation (Supplementary Fig. 28). Turnover frequency (TOF) of the enzyme was 0.036 s⁻¹, which is relatively lower than that of the LiPH₈ enzyme reported in the literature, due to the rate of lignin conversion being limited by H₂O₂ concentration (Supplementary Fig. 29). These results show that TOF can be controlled in a stable manner by adjusting the H₂O₂ generation speed of the photo-electro-biochemical system.

**Photo-electro-biochemical biopolymer synthesis.** The three different types of reactors were also applied for the biopolymer synthesis using conifer alcohol, which is one of the three major units of lignin (Supplementary Fig. 30v). Because current methods of polymer production depend on petrochemicals and, thus, have unavoidable negative environmental effects, polymer synthesis using the abundant, carbon neutral, and recalcitrant lignin biomass is a promising alternative. In the three-compartment photo-electro-biochemical system, biopolymer synthesis reaction was performed with horseradish peroxidase (HRP) biocatalyst with in situ solar H₂O₂ generation at ambient temperature and pressure (Fig. 4a). The conversion efficiency of conifer alcohol monomer and the yield of the biopolymer were 98.3% (Fig. 4b) and 73.3% (Fig. 4c), respectively.

This polymer was comprised of the common linkages between conifer alcohol molecules, such as β-O-4, β-β and β-5, and has a number average molecular weight of 1103, which were confirmed by two-dimensional-nuclear magnetic resonance spectroscopy (2D-NMR, Supplementary Fig. 31), and gel permeation chromatography, respectively.

Unlike the three-compartment system, the single-compartment and two-compartment systems yielded negligible amounts of biopolymers at the end of the reaction (Fig. 4f, i) but a large number of by-products were formed (Supplementary Figs. 32 and 33), although >90% of the monomeric substrate was consumed during the dehydrogenative polymerisation reaction (Fig. 4e, h). Because the HRP biocatalyst shows good stability against O₂ bubbling and solar irradiation (Supplementary Fig. 34), the low selectivity for polymerisation may originate from the generation of highly reactive •OH and •O₂⁻ reaction intermediates on the TiO₂ photocatalyst surface (Fig. 4d). These ROS can lead to further oxidation or the disproportionation of phenolic radicals rather than radical coupling and selective polymerisation (Supplementary Fig. 30). Thus, the majority of the products in the single-compartment reactor are soluble organic compounds rather than biopolymers (Supplementary Figs. 32 and 33). In the case of the two-compartment cell, even though •OH radicals do not interrupt the biochemical reactions, the presence of •O₂⁻ radicals and O₂-coordinating cobalt porphyrin form a superoxide-like structure, which can disrupt the polymerisation reaction/biopolymer formation (Fig. 4g). This leads to the formation of a significant amount of soluble by-products, although the amount produced is lower than that produced in the single-compartment system (Supplementary Figs. 32 and 33). A variety of by-products were detected in the GC–MS analysis (Supplementary Figs. 32 and 33). This indicates that the formation of undesirable highly reactive •O₂⁻ and •OH radicals in these systems resulted in low-molecular-weight by-products rather than selective polymerisation.

However, in the three-compartment photo-electro-biochemical system, the interaction between ROS radicals and the substrate was limited by separating the electrocatalytic (cathodic) and biocatalytic compartments with a cellulose membrane, as experimentally demonstrated earlier (Supplementary Figs. 25 and 26). This results in the selective oxidation of phenolic compounds, and successive radical coupling leads to a high polymer yield. Control reactions performed in the absence of the HRP biocatalyst using all three systems showed no polymer product formation, demonstrating the pivotal role of biocatalyst for biopolymer synthesis (Supplementary Figs. 35–37).

**Discussion**

In summary, we have demonstrated that a compartmented photo-electro-biochemical system that integrates a photocatalyst, an electrocatalyst, and a biocatalyst, is effective for selective and stable lignin dimer valorisation under solar irradiation without the need for any additional bias or sacrificial agent. In this design, the placement of appropriate membranes as separators between cells protects the biocatalyst from detrimental conditions generated during the reaction, thus preserving its stability and activity. This photo-electro-biochemical system can catalyse lignin dimer cleavage with a 93.7% conversion efficiency and 98.7% selectivity, which far surpasses those of single-compartment (37.3% and 34.8%) and two-compartment (25.0%, 48.1%) systems. The system was further applied for sustainable polymer synthesis using a lignin monomer, conifer alcohol, with a 73.3% yield and 98.3% of conversion efficiency; however, the polymer yields of the single-compartment and the two-compartment systems were only ca. 0% and 8.6%, respectively. This unassisted selective lignin valorisation technology could convert waste lignin to value-added aromatics and polymer without the need for any additional energy and chemicals, possibly overcoming the problems associated with
current biomass upgradation, such as its low cost effectiveness and limited processing technology. We believe that the development and scaling-up of this technology will be a milestone for the replacement of petrochemicals with biochemicals.

**Methods**

**Preparation of TiO2 photoanode.** A rutile TiO2 nanowire film was hydrothermal grown on FTO glass. First, 15 mL of hydrochloric acid (35%, Samchun Chemical) was diluted with 15 mL deionised (DI) water and mixed with 0.5 mL titanium (IV) butoxide (97%, Aldrich) in a 100 mL beaker. This clear solution and a clean FTO glass substrate were transferred to a Teflon-lined stainless-steel autoclave (125 mL). The sealed autoclave was heated in an oven at 150 °C for 5 h and then cooled to room temperature (RT) slowly. After rinsing with DI water, calcination was performed at 550 °C for 5 h to increase the crystallinity. Finally, the sample was annealed in a hydrogen atmosphere at 350 °C for 30 min. In addition, an epoxy resin was added behind the TiO2 electrode to block the light transmitted through the TiO2 photoanode. A planar TiO2 film was made on FTO substrate using a radiofrequency sputtering device (SRN-120, SORONA) and calcination was performed at 550 °C for 5 h.

**Synthesis of acid-treated carbon nanotubes (AT-CNT).** Before catalyst synthesis, the CNTs were acid-treated to remove metallic impurities. First, 10.0 g of multi-walled CNTs (MR99, Carbon Nanotech Co., LTD) and 715 g of 6 M HNO3 (diluted from 60 wt% HNO3, Samchun Chemical) were mixed and stirred at 80 °C for 12 h. The CNT slurry was filtered, washed with copious amounts of DI water, and dried at 60 °C. The HNO3-treated CNTs were subsequently washed with 700 g of 6 M HCl (diluted from 36 wt% HCl, Samchun Chemical) in the same manner.

**Synthesis of the Co-N/CNT catalyst.** First, 0.75 g of AT-CNT and 1.50 g of 5,10,15,20-tetrakis(4-methoxyphenyl)-21H,23H-porphine cobalt(II) (CoTMPyP, 98%, Porphyrin Systems) were mixed in an agate mortar for 15 min. The mixture was heated at 400 °C under 1 L min⁻¹ N2 flow for 3 h (ramping rate: ca. 2.1 °C min⁻¹). The heat-treated powder and 3.75 mL of tetraethyl orthosilicate (TEOS, 98%,
Aldrich) were mixed in the mortar for 5 min. The same volume of formic acid (Aldrich) were mixed in the mortar for 5 min. The same volume of formic acid and 60 °C. The nominal Co loading was 1 wt%.

Characterisation of Co-N/CNT. XPS measurements were performed with a K-Alpha X-ray photoelectron spectrometer (Thermo Fisher Scientific) with a monochromatic Al Kα X-ray (1486.6 eV). Co 2p and N 1s XPS spectra were deconvoluted using the XPSPeak software with the incident X-ray power and the Lorenzian (80%)

Catalyst inks were homogenised using an ultrasonic bath. Then, 6 μL of the catalyst ink on both sides of a carbon paper (0.2 mg cm⁻²) was applied and dried at 60 °C, each for 1 h and at different electrode rotation speeds of 2025, 1600, 1225, and 10 cycles. Electrochemical impedance spectroscopy measurement was carried out in 0.1 M phosphate borate solution at pH 4.5 from 0 to 2.00 V (vs. RHE) at a scan rate of 5 mV s⁻¹.

To measure H₂O₂ yield, the Pt ring potential was held at 1.3 V (vs. RHE) during the LSV measurements, and the H₂O₂ selectivity was then calculated according to the following equation:

\[ \text{H}_2\text{O}_2 \text{ selectivity (}) \% = \frac{200 \times \text{a}}{\text{i} - \text{i}_\text{t}} \]

where \( \text{i} \), \( \text{i}_\text{t} \), and \( \text{N} \) indicate the disk current, the ring current, and the collection efficiency (37%, provided by the manufacturer), respectively. To assess the durability of the catalysts, potential cycling tests were performed between 0.6 and 1.0 V (vs. RHE) with an electrode rotation of 1600 rpm at a scan rate of 50 mV s⁻¹ for 1000 cycles in the O₂-saturated electrolyte. After cycling, the ORR activity was measured in a fresh electrolyte.

Hydrogen peroxide detection. The concentration of H₂O₂ was estimated using the DPD method. Depending on the concentration of produced H₂O₂, the samples were diluted with 0.1 M phosphate borate solution at pH 4.5 or 6.0 in the absence of lignin degradation and biopolymer synthesis, respectively, to avoid exceeding the detection limit of the DPD method. In the case of the powder system, the sample was filtered using a 0.45-μm polytetrafluoroethylene (PTFE) filter (SCLR031ML, Millipore). Furthermore, 0.05 g of NaN₃-diethyl-1,4-phenylene-diamine sulphate (DPD, ≥ 98.0%, Aldrich) was dissolved in 5 mL of 0.1 M H₂SO₄ and stored in the dark at 5 °C. The DPD solution was diluted in 5 mL of DI water and kept at 5 °C. Finally, 2.7 mL of a 0.1 M sodium phosphate buffer (pH 6.0), 0.05 mL of DPD solution, 0.05 mL of POD solution, and 0.2 mL of sample mixture were added. The absorbance of mixed solution at λ = 511 nm was measured using a UV/visible spectrophotometer (UV-2600, Shimadzu).

Detection of hydroxyl/superoxide radicals. The formation of OH⁻ was measured using a fluorescence probe method with coumarin 307. 0.2 mM coumarin (Aldrich) was added to the 0.1 M phosphate borate solution at the anode and enzyme cell in the three-compartment reactor, and the fluorescence spectra were measured using a fluorescence spectrophotometer (Cary Eclipse, Varian) with the excitation wavelength at 352 nm. Coloration of XTT reduction to XTT−formazan was used for OH⁻ detection. For this, 0.1 M phosphate borate solution containing 0.1 mM 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT, Aldrich) was added in the cathode and enzyme cell, and the absorbance spectra were recorded from 650 nm to 350 nm using the UV/visible spectrophotometer.

Enzyme preparation. The LiPH8 synthetic gene, including the seven-residue probe sequence, was synthesised by Bioneer Company (South Korea). The gene coding protein sequence was retrieved from UniProtKB database (P06188). LiPH8 was expressed as inclusion body in Echerichia coli BL21 (DE3) and reactivated through in vitro refolding procedure as previously reported with slight modification. The inclusion body was added into the refolding solution containing 100 mM Tris–HCl pH 8.0, 500 mM sodium acetate, 2 mM CaCl₂, 20 mM HEPES, sodium dodecyl sulphate, and 0.05 M Tris–HCl pH 6.0. The mixture was then incubated with sodium acetate buffer 100 mM at pH 4.0 then pH 6.0 before subjected for purification. The anion-exchange chromatography with Mono Q 5/50 GL column (GE Healthcare Life Sciences Co., USA) was used for purification step in ammonium formate solution containing 0.1 M sodium acetate buffer at pH 6.0 and eluted with a linear gradient of buffer B—sodium acetate 500 mM, pH 6.0. The highest activity fraction of LiPH8 was tested with sodium dodecyl
sulfate–polyacrylamide gel electrophoresis and used for this study. HRP type VI was purchased from Aldrich Co. (USA) and used without any further purification.

Photo-electro-biocatalytic lignin conversion. The overall reaction was performed in an acrylic reactor composed of an anode cell with a quartz window on one side, a cathode cell, and an enzyme cell. In the case of the three-compartment reactor, the anode cell was separated from cathode cell through a Nafion membrane (Nafion® 117, 0.18 mm thick, Aldrich), and the cathode cell was separated from an enzyme cell through a cellulose membrane (Spectra/Por®, 8–8kDa, Spectrum). For the lignin dimer conversion, 0.1 M phosphate borate solution of pH 4.5 was used as the electrolyte. The anode cell, cathode cell, and enzyme cell consisted of H:TiO2 photoanode (1.33 cm²) with 8 mL electrolyte, Co-N/CNT-based cathode (2.0 cm²) with 4 mL electrolyte, and 0.5 mM of lignin dimer and 0.8 μM of LiPH8 in 8 mL electrolyte, respectively. The photoanode and cathode were connected to each other with alligator clips and copper wire as an external circuit. In the case of the two-compartment reactor without an enzyme cell, the cathode cell was composed of the Co-N/CNT cathode, 0.5 mM of lignin dimer, and 0.8 μM of the LiPH8 in 4 mL electrolyte. In the single cell, Degussa P25 TiO2 powder (0.5 mg mL⁻¹) was used as a photocatalyst for H₂O₂ production, 0.5 mM of lignin dimer, and 0.8 μM of the LiPH8 enzyme were added together in 8 mL electrolyte. The photoanode (or P25 photocatalyst) was illuminated using a solar simulator (10500, Abet Technologies) at 100 μW cm⁻² (AM1.5 G). O₂ gas was continuously bubbled into the cathode electrolyte from 20 min before the reaction until the reaction was completed. The bioprocess system was performed in the three kinds of the reactors as depolymerisation of lignin with slight modifications. The reactions were carried out with 0.5 mM of coniferyl alcohol as a substrate and 1.4 μM of the HRP as an enzyme in 0.1 M phosphate borate solution at pH 6.0.

Identification of phenolic compounds. The completed reaction mixture was analysed by high-performance liquid chromatography (HPLC). The HPLC procedure was performed by injecting fractions using an Agilent 1200 HPLC system onto a reverse-phase Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm, Agilent). Gradient separation was performed using 0.1% aqueous trifluoroacetic acid (solvent A) to methanol/acetonitrile (25:75; v/v; solvent B) with the following conditions: analysis time of 15 min flow of 1.5 mL min⁻¹ and column temperature of 30 °C. The gradient programme was as follows: 0 min—15% B, 6 min—60% B, 11.5 min—100% B, and 13 min—0% B. Before injection, the sample was filtered through a hydrophilic 0.2-μm PTFE membrane filter. The products were identified relative to an authentic library of standards based on their retention times and UV absorption spectra. The quantification of the reaction was performed by HPLC based on a linear external standard curve (R² > 0.95) of the respective compound. For the by-products, the phenolic derivatives were extracted with dichloromethane and analysed using a GC–MS (Agilent) with a DB-5MS (60 m x 0.25 μm) column. The oven temperature was elevated from 50 to 280 °C. The product mass spectrum was identified using an authentic library of standards.

Characterisation of the polymer. The polymer was collected by centrifugation at 13,000 rpm within 10 min, washed three times with 2 N HCl solution, and dried under vacuum conditions. The polymer yield was determined by UV analysis at 420 nm based on a linear external standard curve (R² > 0.95) of the respective compound synthesised dehydrogenative polymer from coniferyl alcohol. The dried polymer was dissolved in 0.5 mL DMSO-d₆ before NMR analysis. NMR spectra for an engineered, catalytically active Trp radical that creates the unique reactivity of lignin peroxidase. Proc. Natl Acad. Sci. USA 106, 16084–16089 (2009).

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Author contributions

J.-W.J., Y.H.K., and S.H.J. proposed, designed, and directed the research. J.-W.J., M.K., L.T.M.P., and Y.J.S. conceived the concept of the compartmented photo-electro-biocatalyst system. M.K. and L.T.M.P. prepared the photocatalysts with D.O. and the biocatalysts with T.V.T.N., respectively, and measured the conversion efficiency and selectivity for lignin valorisation. Y.J.S. prepared and characterised the electrocatalysts and measured their performances with the help of J.W. and H.H.K. P.S., J.R., and T.J.S. helped to analyse the data and provided valuable input. M.K., L.T.M.P., Y.J.S., S.H.J., Y.H.K., and J.-W.J. co-wrote the manuscript. All authors read and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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