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1. Introduction

Nowadays, capture,^{1,2} trapping,³ and utilization⁴ of CO₂ are the processes that are widely used for reducing atmospheric CO₂ gas to mitigate global warming.^{1,5} In the cases of capture and trapping, CO₂ is adsorbed on the active sites and transported to the active storage site, respectively.^{1,6} In the utilization process, CO₂ gas can be utilized in the transformations to useful chemicals or fuels, which has been attempted via four major methods: chemical,7 photochemical,8 electrochemical,9 and enzymatic conversion.¹⁰ In particular, for the production of fuels, it is important to set up methods that require no additional CO₂-generating power sources.^{10,11} Thus, enzymatic conversion for the production of solar fuels has significant advantages over conventional techniques.10 Natural leaves are good example of this enzymatic system, in which the conversion of CO₂ gas to glucose is performed with the aid of water under photocatalytic activity.^{12,13} Thus, for the production of solar fuels using renewable energy sources that mimic the process of a natural leaf to mitigate global warming, photoinduced enzymatic reaction will become one of the important techniques.

The photoreaction system for the production of solar fuels is a photoredox system with a hybrid of a photosensitizer and an

Photoinduced enzymatic conversion of CO₂ gas to solar fuel on functional cellulose nanofiber films

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Functional cellulose nanofiber films for the production of solar fuel from CO_2 gas were prepared by immobilizing dendrimer and porphyrin derivative and loading an electron donor and enzymes (formate, aldehyde, and alcohol dehydrogenases) on the films. Porphyrin derivative is a photosensitizer, and the dendrimer plays the role of a reservoir of guest gases and carrier of electron/proton in addition to acting as an intermediate for the binding of porphyrin derivative on the nanofiber. However, after laser irradiation, whereas the remaining amount of gas on the film without an electron donor and enzymes was almost half of that without laser irradiation, it on the films loaded with an electron donor and enzymes was almost equal to or slightly higher than that without laser irradiation, whereas it was almost equal to or slightly higher the conversion of CO_2 gas under laser irradiation. The conversion efficiency from CO_2 to formic acid was only half because of the fact that only the adsorbed CO_2 in the vicinity of the reaction system reacted; however, the conversion efficiencies from formic acid to formaldehyde to methanol were ~80 and ~90 wt%, indicating the successful stepwise conversion by the photoinduced enzymatic reaction.

electron carrier,¹⁴ where visible light is irradiated to activate photosynthesis. Some studies on the visible light-induced CO₂ reduction with enzymes have been reported.15-20 However, in all the trials, either methanol was not produced in useful yields or the conversion of light to chemical energy was not achieved.13,14 Thus, the photoinduced enzymatic conversion of CO₂ to methanol has not been reported to date because of the difficulty in generating and transferring the electrons in the system to accomplish the reaction. It has been speculated that a photoinduced enzymatic system can sequentially reduce CO2 to formic acid,^{13,21} formaldehyde,²² and methanol^{13,22} using formate, aldehyde, and alcohol dehydrogenases, respectively, if an adequate electron donor is used in the enzymatic reduction system, where six electrons are required to be generated and transferred to accomplish CO2 reduction to methanol.11,23 Additionally, in all the previously reported cases, the reduction was performed in aqueous solutions, dissolving CO₂ gas; thus, the direct gas conversion to methanol was a challenging task.

We previously reported the development of systems for the adsorption and storage of CO_2 gas on dendrimerincorporated organoclays^{6,24} and organoclay-incorporated 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-oxidized cellulose nanofiber (TOCNF) films.² TOCNF films are porous²⁵ and hydrophilic in nature;² moreover, as a promising biomaterial with many available binding sites,² these films can be used as a substrate in reactions. In addition, dendrimers possess highly functional terminal groups and are adaptable to gas adsorption^{6,24} as well as to the electron carrier.²⁶ Additionally,



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porphyrin derivatives are common photosensitizers because they strongly absorb light in the 400–450 nm region.²⁷ The TOCNF film can incorporate a photosensitizer and an enzymeloaded electron carrier to construct a photoinduced enzymatic system. Herein, we developed a photoinduced enzymatic reaction film, having a porphyrin derivative, a dendrimer, and enzymes, for the sequential synthesis of formic acid, formaldehyde, and methanol from CO₂ gas under laser light irradiation, and the formation of solar fuel was assessed.

2. Experiments

2.1. Materials

Abies sliced wood (softwood) and TEMPO free radical were obtained from Canary Corporation (Taiwan) and Acros organics (USA), respectively. Enzymes (formate dehydrogenase (FDH) from Candida boidinii, aldehyde dehydrogenase (AlDH) from pseudomonas, and alcohol dehydrogenase (ADH) from saccharomyces) were purchased from Sigma-Aldrich (USA). Amine-terminated fourth generation poly(amido amine) (PAMAM) dendrimer (Den, MW 14 213), n-hydroxysuccinimide (NHS), and an electron donor (β-nicotinamide adenine dinucleotide 2'-phosphate-reduced tetrasodium salt hydrate (NADPH)) were also purchased from Sigma-Aldrich (USA). Tetrakis-(4-carboxyphenyl)porphyrin (TCPP) was a product obtained from the Tokyo Chemical Industry Co., Ltd. (Japan). Colorimetric reagents (sodium nitroprusside, L-tryptophan, and potassium ferricyanide) and 1-(3-dimethylaminopropyl)-3ethylcarbodiimide hydrochloride (EDC) were purchased from Acros organics (USA). Ultrapure water (resistivity of $18.2 \text{ M}\Omega \text{ cm}$) was obtained from a Yamato Millipore WT100. All other commercial chemicals were of analytical grade and used without further purification.

2.2. Instruments and techniques

A QSONICA Q700 ultrasonicator with a half-inch tip was used to reduce the size of the fiber to the nanometer size. Ultraviolet (UV)-visible absorption spectra were obtained using a Jasco V-670 series spectrometer with a 1 mm or 1 cm quartz cell. The adsorption–desorption curves of CO₂ gas were obtained *via* a thermogravimetric analysis (TGA) instrument (Q500 TA) at the scan speed of 10 °C min⁻¹ under a pure CO₂/N₂ gas flow in the sample chamber. The photoreaction was carried out using 488, 633, and 785 nm wavelength laser sources of an argon laser (Stellar-Pro 488/50 (Modu-Laser, LLC, USA)), a HeNe laser (15-LHP-925-249 (Melles Griot, USA)), and a Littman/Metcalf Diode Laser System-Lion (Pilot PZ 500 (Sacher Lasertechnik, Germany)), respectively. The powers of the 488, 633, and 785 nm lasers measured using a Solo PE power and energy meter (Gentec-EO, Canada) were 13.5, 12.8, and 15.8 mW, respectively.

2.3. Preparation of the Den-TCPP-TOCNF film

TOCNF was same as that previously prepared through dewaxing and TEMPO oxidation processes, as described in a previous report.² In brief, the soft abies wood was dewaxed by refluxing with toluene/ethanol, delignifying using sodium chlorite, and thorough washing. The obtained never-dried pulp was oxidized in the presence of sodium bromide, sodium hypochlorite, and TEMPO and reduced by sodium borohydride, followed by filtration of COONa-functionalized TOCNF. Finally, the nanofiber formation was accomplished by ultrasonicating the slurry of the reduced COONa-functionalized TOCNF.

TCPP and dendrimer were immobilized onto TOCNF through chemical bonding by the amidation reaction using the condensation reagents EDC and NHS; the amine-terminated dendrimer with carboxyl-functionalized materials was first bound with TCPP and subsequently with TOCNF to prepare the Den-TCPP-TOCNF.²⁸ Briefly, an aqueous solution of EDC : NHS (1:1 molar ratio) was mixed with an aqueous TCPP solution, and then, an aqueous solution of dendrimer (1:1 molar ratio for Den: TCPP) was added for amidation. The product Den-TCPP purified by centrifugation was immobilized onto TOCNF with different weight ratios through the same amidation process using the condensation agents EDC and NHS. The product Den-TCPP-TOCNF was purified by centrifugation and washing with water. The Den-TCPP-TOCNF film was obtained by vacuum-drying the residue on filter paper after filtration for 24 h and by peeling off from the filter paper. TOCNF film displayed a network texture in the AFM image similar to the previously reported film.²

2.4. Enzyme loading on the Den-TCPP-TOCNF film and adsorption/desorption experiments on it

One unit of FDH activity was defined as the amount of enzyme required to convert 1.0 µmol CO2 to formic acid in 1 min in the presence of NADPH at pH 7.6 and room temperature.14 Similarly, one unit of AlDH and ADH activity was defined as the amount of enzyme required to convert 1.0 µmol formic acid to formaldehyde and 1.0 µmol formaldehyde to methanol, respectively, in 1 min in the presence of NADPH.13,29 To obtain these materials on the Den-TCPP-TOCNF film, FDH, AlDH, and ADH enzymes were loaded onto the Den-TCPP-TOCNF film as follows. Based on the CO₂ adsorption capacity of the Den-TCPP-TOCNF film, the calculated amount (unit) of enzymes was diluted with a 0.1 M bis-tris buffer solution (pH 7) and dropped on the entire film by selecting a single enzyme for the single reaction and multiple enzymes for the multiple reactions. After the dropping process, the films were quickly vacuum-dried and immediately utilized for CO2 gas adsorption in the TGA analysis.

Following previous reports,^{2,6} the adsorption experiment of CO_2 on the Den–TCPP–TOCNF films, which were loaded with enzymes and NADPH, was carried out for 300 min at room temperature using a TGA instrument with a N₂ gas flow (balance chamber) and CO_2 (pure) gas flow (sample chamber) at the flow rates of 40 and 60 mL min⁻¹, respectively. In the desorption experiment, films, which adsorbed CO_2 , underwent CO_2 desorption under a N₂ flow in the sample chamber.

2.5. Designing the photoreaction system

A quartz cell with a 1 cm pass length was used for the photoreaction, and UV spectral analysis was directly performed on it.

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The photoreactor cell maintained the humidity using drops of water kept at its bottom. Thus, this humid chamber could prevent the degradation of enzymes or organic molecules due to overheating during laser irradiation. An enzyme-loaded Den-TCPP-TOCNF film was hung by a holder inside the photoreactor cell, and laser light was irradiated on the film to activate the photoreaction. To determine the conditions (wavelength and time) of laser irradiation, a pre-experiment was carried out in a photoreactor cell, where an NADPH solution (5 mmol) was used. Conversion of CO₂ by enzyme was directly calculated by concentration variation from NADPH to NADP⁺ through UVvisible absorption spectrometry. For the photoreaction in the gas state, NADPH as well as enzymes were loaded on the Den-TCPP-TOCNF-film. CO2-adsorbed Den-TCPP-TOCNF film loaded with enzyme and NADPH was hung in a humid reactor, followed by laser light irradiation for 30 min. After the irradiation process, the conversion of CO₂ on the film was evaluated from N_2 adsorption *via* TGA.

2.6. Detection of the reaction products

Products obtained after photoinduced enzymatic reaction were extracted by dipping a reaction film in water (2 mL). Products in the extracted solutions were colorimetrically detected using different indicators. For the detection of formic acid,³⁰ L-tryptophan (25 mM) was mixed with concentrated hydrochloric acid (6 M) and a dimethyl sulfoxide (0.2% volume/volume) solution, and the mixed solution was diluted with water to double volume and added dropwise to the extracted solution. The mixture was reacted at 50 °C for 3 h and analyzed using a UV-visible absorption spectrometer. For the detection of formaldehyde,^{31,32} ammonium acetate (150 g), acetic acid (3 mL), and acetyl acetone (2 mL) were mixed, and the mixture was diluted to 1 L with water. The diluted mixture was mixed with an equal amount of extracted solution containing products and reacted at 40 °C for 30 min. The reaction solutions changed color (violet blue, detectable by naked eye) and were analyzed using a UVvisible absorption spectrometer. For the detection of methanol,33 sodium nitroprusside (10% mass/volume), potassium ferricyanide (10% mass/volume), and sodium hydroxide (5% mass/volume) were mixed, and the mixture was kept in the dark at 4 °C. The mixture diluted with water was mixed with a methanol-containing extracted solution and kept at room temperature for 15 min. A color change (yellow to orange) was observed with the naked eye, and the reaction solutions were analyzed using a UV-visible absorption spectrometer. To calculate the conversion amounts, the calibration curves were prepared over the concentration range, where a linear relationship of the Beer-Lambert equation was obeyed.

3. Results and discussion

3.1. Characterization of the Den-TCPP-TOCNF film and adsorption of CO₂ gas on it

The photoinduced enzymatic reaction system for CO₂ conversion requires a photosensitizer and an electron carrier for electron transfer in the system.^{13,14} Moreover, the system should

be activated by visible light irradiation. Thus, TCPP and dendrimer were immobilized as a photosensitizer and an electron carrier, respectively, on the TOCNF film. When a Den-TCPP (1:1 molar ratio) hybrid was prepared from a PAMAM dendrimer and TCPP by amidation with condensing reagents, a pale pink color of the Den-TCPP solution was observed instead of a pale dark green color of a TCPP solution (see inset of Fig. 1). Fig. 1 shows the UV-visible absorption spectra of the solutions of the dendrimer, TCPP, and the Den-TCPP hybrid. While the dendrimer and TCPP displayed an absorption band at 280 and 414.5 nm, respectively, an absorption band for their hybrid was observed at 421.5 nm. This red-shift of the absorption band of TCPP can be attributed to the formation of the amide linkage, indicating preferable electron transfer.

The immobilization of Den–TCPP on TOCNF to prepare Den– TCPP–TOCNF was carried out by chemical binding. To confirm this immobilization, the weight loss was examined using TGA, as shown in Fig. 2. While three weight loss steps were observed at 160, 295, and 548 °C for the dendrimer, two weight loss steps were observed at 430 and 895 °C for TCPP and at 300 and 450 °C



Fig. 1 UV-visible absorption spectra of aqueous solutions of PAMAM dendrimer, TCPP, and Den–TCPP hybrid. Insets are the images of the solutions of TCPP and Den–TCPP hybrid.



Fig. 2 TGA of PAMAM dendrimer, TCPP, TOCNF, and Den–TCPP– TOCNF (0.6 g (Den–TCPP) per g (TOCNF)).

for the TOCNF film. In the case of the Den–TCPP–TOCNF (0.6 g (Den–TCPP) per g (TOCNF)) film, four weight loss steps were observed at 100, 283, 542, and 890 °C. While the weight loss below 200 nm corresponds to the removal of the coexisting water and/or contaminant molecules, other losses correspond to the decomposition of the dendrimer, TCPP, and TOCNF.

Amine-terminated PAMAM dendrimer, which consists of branched units, is a capable candidate for CO₂ capture and storage.^{2,6} Because of these benefits, dendrimers can be incorporated as storage and electron transfer moieties in a device. The CO₂ adsorption on Den-TCPP-TOCNF films with different immobilized contents of Den-TCPP is shown in Fig. 3. In common, after the films were exposed to CO₂ gas, CO₂ adsorption started and slowed down with the occupation of gas molecules on the film; moreover, adsorption at high Den-TCPP content did not reach saturation even after a reaction time of 300 min. The adsorption capacity of the TOCNF film without Den-TCPP was very less because of limited availability of the active binding sites on TOCNF,6 whereas on the TOCNF film, it increased with an increase in the Den-TCPP content because of available binding sites of coexisting dendrimers. The films with adsorbed CO₂ gas were used for the photoreaction.⁶ Note that the adsorption of CO₂ gas on the Den-TCPP-TOCNF films is less than that on the dendrimer-clay (organoclay) powder and organoclay-TOCNF films.2,6 According to the kinetic analysis of the CO₂ gas adsorption,²⁴ there must be three adsorption sites on organoclays: sites of clay surface, clay interlayer, and dendrimer. In the case of the Den-TCPP-TOCNF film in the present investigation, the first two binding sites were lacking and only the dendrimer binding site was present to adsorb the CO₂ gas.

3.2. Effects of wavelength and time of laser irradiation on the activity of photoinduced enzymatic reactions in an aqueous medium



To determine an adequate laser source and its irradiation time, the photooxidation reaction on the enzyme (FDH)-loaded Den– TCPP–TOCNF film, in which CO₂ gas was adsorbed, was performed under visible-light irradiation from the laser sources in

Fig. 3 Time-dependent CO₂ adsorption on the Den–TCPP–TOCNF films at different Den–TCPP contents.

an aqueous NADPH solution. The oxidation reaction of NADPH to NADP⁺ in an aqueous solution in the presence of an enzyme and adsorbed CO2 gas on the films was carried out under irradiation of laser lights with different irradiation wavelengths (488, 633, and 785 nm), and the conversion of NADPH to NADP⁺ was observed by UV-visible absorption spectroscopy. The changes in the absorption spectra obtained in water at different irradiation times of a 488 nm laser are shown in Fig. 4. The absorption bands at 260, 340, and 421 nm are attributed to the presence of NADP⁺, NADPH, and TCPP, respectively. The decreased absorbance of NADPH and the increased absorbance of NADP⁺ indicate that the oxidation reaction took place within the irradiation time, whereas the invariable absorbance of the 421 nm band indicates no variation of TCPP on laser irradiation. A similar tendency was observed even with irradiation by 633 and 785 nm lasers. The evaluated % oxidation ((A(t = 0) - $A(t)/A(t = 0) \times 100$ at the irradiation time t is plotted as a function of irradiation time in Fig. 5. The % oxidation increased with the increasing irradiation time, and the



Fig. 4 UV-visible absorption spectra, as a function of irradiation time of 488 nm laser, of an NADPH/enzyme(FDH)-loaded Den–TCPP–TOCNF film immersed in an aqueous NADPH solution.



Fig. 5 The % oxidation, as a function f irradiation time using different lasers, of an NADPH/enzyme(FDH)-loaded Den-TCPP-TOCNF film (0.6 g (Den-TCPP) per g (TOCNF) at 1 : 1 molar ratio for den : TCPP) immersed in an aqueous NADPH solution.



Fig. 6 Time-dependent CO_2 gas adsorption (left) and desorption after 30 min laser (488 nm) irradiation (right) on the Den-TCPP-TOCNF films (0.6 g (Den-TCPP) per g (TOCNF) at 1 : 1 molar ratio for Den : TCPP). (a) Without NADPH/enzymes, (b) with NADPH/FDH, (c) with NADPH/(FDH + AlDH), and (d) with NADPH/(FDH + AlDH).

conversion of NADPH to NADP⁺ was highest under the irradiation of blue laser light at 488 nm. This suggests that the NADPH conversion is affected by the absorption band (421 nm) of the photosensitizer (TCPP) because the laser wavelength of 488 nm is near to the excitation wavelength of TCPP. Hereinafter, only the blue laser light was used.

3.3. Conversion of CO₂ gas on the NADPH/enzymes-loaded Den-TCPP-TOCNF films

Different enzymes (FDH, AlDH, and ADH) were treated stepwise along with NADPH on the Den-TCPP-TOCNF films to prepare photoinduced enzymatic systems loaded with FDH, FDH + AlDH, and FDH + AlDH + ADH. Thus, photoinduced conversion of CO₂ was carried out on the NADPH/enzymes-loaded Den-TCPP-TOCNF films in a humid photoreactor cell. After the adsorption of CO₂ was performed on the enzyme/NADPHloaded Den-TCPP-TOCNF film, laser light at 488 nm was irradiated for 30 min on the films with adsorbed CO₂, and N₂ gas adsorption (CO₂ gas desorption) was carried out. The obtained profiles of different enzyme combinations loaded on the Den-TCPP-TOCNF films are presented in Fig. 6, which includes a profile of the Den-TCPP-TOCNF film without loaded NADPH and enzymes. The adsorption and desorption processes exponentially occurred with time, that is, they rapidly changed at the beginning and then approached and reached steady values.

The adsorbed (a) and remaining (r(with)) amounts of gases on the films at the longest time are listed in Table 1 and plotted in Fig. 7, which include the amount of gases on a pristine TOCNF film. Similar CO₂ desorption experiments without laser irradiation were carried out on the films, and the remaining (r(without)) amounts of gases are also listed and plotted. The adsorbed amount of CO2 on the TOCNF film without Den-TCPP and NADPH/enzymes was less, and CO2 was almost completely desorbed from the TOCNF film; moreover, the amount of adsorbed CO₂ increased with the immobilization of Den-TCPP and loading of NADPH/enzymes on the TOCNF film because new binding sites for CO₂ in the adsorbents were added in addition to the binding site on the TOCNF.24 However, almost half of the adsorbed CO₂ remained on the NADPH/enzymeloaded Den-TCPP-TOCNF films after the desorption process without laser irradiation. This behavior was similar to that reported in previous studies stating that CO₂ molecules were adsorbed on the organoclays or organoclay-TOCNF films, but



Fig. 7 Gas amounts on TOCNF, Den–TCPP–TOCNF, and NADPH/ enzymes-loaded Den–TCPP–TOCNF films. (a, black) Adsorbed, (r(without), red) remaining after being desorbed without laser irradiation, and (r(with), blue) remaining after laser irradiation and desorption.

they only remained on the dendrimer binding site and not on the clay and TOCNF binding sites after the desorption procedure.²⁴

Note that the gas remaining on the Den-TCPP-TOCNF film without NADPH/enzymes was less for the condition of laser irradiation than that for the case without irradiation. This behavior can be interpreted by the fact that CO₂ adsorbed on the dendrimer may be partly desorbed (almost half) because of the irradiation of the energy-rich laser (comparison between A1 and A2 shown in Scheme 3). In contrast, for the NADPH/enzymesloaded Den-TCPP-TOCNF films, the remaining gas amount after laser irradiation was almost equivalent to or slightly higher than the remaining gas amount without laser irradiation. These results suggest that the products of the photoinduced enzymatic reaction on the NADPH/enzymes-loaded Den-TCPP-TOCNF films may be accumulated on the films (comparison between B1 and B2 shown in Scheme 3).

Table 1 Amounts of adsorbed CO₂, remaining CO₂, remaining gas, formic acid, aldehyde, and methanol on different films. Amount (ppm) was calculated per 1 g of film

Film		Adsorbed CO ₂ (ppm)	Remaining CO ₂ (without irradiation) (ppm)	Remaining gas (with irradiation) (ppm)	HCOOH (ppm)	HCHO (ppm)	CH₃OH (ppm)
TOCNF		420	20				
Den-TCPP-	No NADPH/enzyme	5100	2500	1000			
TOCNF	NADPH/FDH	6800	2800	2900	446	11	35
	NADPH/(FDH + AlDH)	8100	3000	3200	111	218	46
	NADPH/(FDH + AlDH + ADH)	9200	3500	3800	89	29	203

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3.4. Identification of the products from the adsorbed CO_2 gas

According to the procedure for the detection of the extracted part of each product formed by the photoinduced enzymatic reaction on the NADPH/enzymes-loaded Den-TCPP-TOCNF films, the color changes of the extracted solutions on adding an indicator were visualized by naked eye, and the absorption spectra of the solutions were obtained, as shown in Fig. 8. The absorbance of the colorimetric indicators for formic acid, formaldehyde, and methanol was obtained at 580, 413, and 481 nm, respectively, for the cases of the enzymes FDH, FDH + AlDH, and FDH + AlDH + ADH on the Den-TCPP-TOCNF films.

The calculated conversion amounts are listed in Table 1. For the NADPH/enzymes-loaded Den–TCPP–TOCNF films, formic acid of 446 ppm g^{-1} (film) was detected on the FDH-loaded film, but it was less for the other enzymatic systems. The amount of formaldehyde was 218 ppm g^{-1} (film) on the film with FDH + AlDH enzymes, but the presence of formaldehyde was less on

other enzyme-immobilized films. When the enzymes FDH + AlDH + ADH were loaded on the film, the amount of methanol (203 ppm g^{-1} (film)) was larger than that in other enzyme systems. The results are reasonable when the stepwise enzyme reaction is considered. That is, FDH, AlDH, and ADH convert CO_2 to formic acid, formic acid to formaldehyde, and formaldehyde to methanol, respectively. The \sim 80 wt% of formic acid produced by ADH was converted to formaldehyde by AIDH, and \sim 90 wt% of formaldehyde was converted to methanol by ADH. Therefore, it can be inferred that the efficiency of the photoinduced enzymatic reaction is sufficiently high. Table 1 also lists the remaining CO2 amount on the Den-TCPP-TOCNF film without NADPH/enzymes, which was laser-irradiated. Despite the remaining amount of 1000 ppm of CO2, the yield of product of the first enzymatic reaction was almost half. This result may be due to the fact that only the adsorbed CO₂ in the vicinity of Den-TCPP might have reacted because the photosensitizer (TCPP) and the electron carrier (dendrimer) are indispensable for the photoinduced enzymatic reaction.



Fig. 8 Color (left) and UV-visible adsorption spectra (right) of solutions extracted after photoinduced enzymatic reaction (488 nm laser irradiation for 30 min) on the NADPH/enzymes-loaded Den-TCPP-TOCNF films (0.6 g (Den-TCPP) per g (TOCNF) at the 1 : 1 molar ratio for Den : TCPP). (a) FDH, (b) FDH + AlDH, and (c) FDH + AlDH + ADH. (1, red) Formic acid, (2, black) formaldehyde, and (3, blue) methanol.

3.5. Mechanism of CO₂ gas conversion in the photoinduced enzymatic system

Based on the results obtained in this investigation, the reaction scheme of the solar fuel formation on the NADPH/enzymesloaded Den-TCPP-TOCNF films is illustrated in Scheme 1. The reactions occurring on the NADPH-loaded Den-TCPP-TOCNF films are described as below.

 $H_2O \rightarrow (\frac{1}{2})O_2 + 4e^- + 2H^+$ $TCPP^+ + 2e^- + H^+ \rightarrow TCPP$ $TCPP + h\nu \rightarrow TCPP^*$ $TCPP^* \rightarrow TCPP^+ + 2e^- + H^+$ $Den-NH_3^+ + 2e^- + H^+ \rightarrow Den-NH_2$ $Den-NH_2 \rightarrow Den-NH_3^+ + 2e^- + H^+$ $NADP^+ + 2e^- + H^+ \rightarrow NADPH$ $NADPH \rightarrow NADP^+ + 2e^- + H^+$

In this reaction system, TCPP was a photosensitizer and it was excited with visible light (488 nm laser). During the reaction, water molecule worked as an electron donor to TCPP⁺ and it was oxidized to O2 molecules.12,34 The dendrimer played the role of an electron carrier to transfer electrons from TCPP to NADPH. NADPH was converted to NADP⁺ and the electrons were transferred to the enzymatic system. When the system contained a single enzyme (FDH), CO₂ gas was converted to formic acid molecules, whereas in the multiple enzymatic systems of FDH + AlDH and FDH + AlDH + ADH, formic acid was sequentially converted to formaldehyde and methanol, depending on the electron and proton transfer in the system. Thus, note that the hybrid of the TOCNF film with the dendrimer and TCPP is a suitable artificial photoreaction device that mimics plant photosynthesis. The artificial photoreaction system was modified to produce solar fuel by loading adequate enzymes because the production of solar fuel depends on the presence of electron and proton carriers in the system.

CO₂ molecules were preferably captured on the dendrimers immobilized on a cellulose nanofiber scaffold by the formation of carbamate species between CO₂ and the amine-terminal of the dendrimer.^{2,35} TCPP was chemically bound on the dendrimer, and NADPH and the enzymes preferably loaded on the dendrimer may be involved in the enzymatic reaction, as seen in



Scheme 1 Schematic of the photoinduced enzymatic reaction on the NADPH/enzyme-loaded Den-TCPP-TOCNF films.



Scheme 2 Schematic of the Den-TCPP-TOCNF film and photoinduced enzymatic reaction on the NADPH/enzymes-loaded Den-TCPP-TOCNF film.





Scheme 3 Schematic of the gas state on the Den-TCPP-TOCNF and NADPH/enzymes-loaded Den-TCPP-TOCNF films without and with laser irradiation.

Scheme 2. In this location, the photo energy gained by the photosensitizer (TCPP) was transferred to electrons, which were then transferred to the electron carrier (dendrimer) and NADPH, and the electrons from NADPH were provided for the enzymatic reaction for the conversion of CO_2 .

4. Conclusions

In this study, an artificial leaf, mimicking a natural leaf, for photoreaction was modified to establish the photoinduced enzymatic reaction for solar fuel production from CO_2 gas. To store CO_2 for its conversion to organic products, a dendrimer with a suitable storage site for CO_2 (ref. 2, 6 and 24) was immobilized along with a photosensitizer (TCPP) on the TOCNF film (see Scheme 2). To optimize the storage amount of CO_2 , the dendrimer should be highly immobilized on the TOCNF films, whereas one TCPP is enough on one dendrimer. Moreover, to construct the reaction system, NADPH as an electron donor and enzymes (FDH, AlDH, and ADH) were loaded on the Den-TCPP/ TOCNF film. For different visible light sources such as 488, 633, and 785 nm lasers for photoinduction, oxidation of CO_2 on the FDH enzyme was the highest under 488 nm light irradiation because this irradiation wavelength nearly matched with the excitation wavelength of TCPP; moreover, 488 nm is a shorter wavelength with high energy for the conversion reaction.

Our ultimate goal was the development of a system (or a device) for solar fuel production from CO_2 gas, and accordingly, different enzymes were loaded on a Den-TCPP-TOCNF film (see Scheme 2). Although adsorption of CO_2 occurred on the dendrimer and TOCNF, the remaining CO_2 molecules after desorption were located on the dendrimer.² However, the amount of gas on the dendrimer of the Den-TCPP-TOCNF film without NADPH/enzymes decreased under laser irradiation because of photo removal of CO_2 , and the amount of remaining gas on the NADPH/enzymes-loaded Den-TCPP-TOCNF films after irradiation was close to or slightly higher than that on the films without irradiation. This indicated that the conversion of CO_2 occurred on the NADPH/enzymes-loaded Den-TCPP-TOCNF film. The conversion efficiency to solar fuel was about half for formic acid, ~80 wt% for formaldehyde, and ~90 wt% for methanol. Thus, the system developed in the present study can be effective for the production of solar fuel from CO_2 gas, and this is the first report on the direct conversion of CO_2 gas to methanol.

Moreover, the utilization of a cellulose nanofiber film as a substrate for immobilizing functional molecules (photosensitizer, electron carrier, electron donor, and enzymes) has various advantages: the cellulose nanofiber is a renewable natural resource, cheap, naturally degradable, and easily functionalized. It is a fine fiber and can be fabricated as lightweight and flexible transparent films. The cellulose nanofiber film does not ravel out and swell even in water, and it is reusable. In fact, we fabricated functional cellulose fiber films, which enabled antimicrobial activity and gas capture in air and chemical reaction in solution by means of the characteristics of the films described above.^{2,36–38} Thus, the cellulose nanofiber film can be prepared and cut into any size and shape. Thus, the cellulose nanofiber film is a promising candidate for a new type of substrate because of these advantages.

Recently, some researchers have reported CO_2 -to-solar fuel conversion using different reaction mechanisms with a photocatalyst, enzyme-electrochemistry, and photo-thermochemical reaction.^{39–42} Photoinduced enzymatic reactions have also been tried for different purposes^{43–45} including CO_2 -to-solar fuel conversion in water.^{46,47} Cellulose nanofibers have been utilized as a matrix for immobilizing materials^{48,49} and for the composite formation of cellulose nanofiber with reduced graphene oxide.⁵⁰ Thus, the present CO_2 -to-solar fuel conversion system to methanol in air *via* a photoinduced enzymatic reaction on a cellulose nanofiber matrix is our original and valuable since the system has additional merits of easy to removable and reusable.

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References

- 1 D. Y. C. Leung, G. Caramanna and M. M. Maroto-Valer, *Renewable Sustainable Energy Rev.*, 2014, **39**, 426–443.
- 2 K. J. Shah and T. Imae, *Biomacromolecules*, 2016, **17**, 1653–1661.
- 3 C. M. White, B. R. Strazisar, E. J. Granite, J. S. Hoffman and H. W. Pennline, *J. Air Waste Manage. Assoc.*, 2003, 53(6), 645– 715.
- 4 K. M. K. Yu, I. Curcic, J. Gabriel and S. C. E. Tsang, *ChemSusChem*, 2008, 1, 893–899.
- 5 C. H. Yu, C. H. Huang and C. S. Tan, *Aerosol Air Qual. Res.*, 2012, **12**, 745–769.
- 6 K. J. Shah, T. Imae and A. Shukla, RSC Adv., 2015, 5, 35985– 35992.

- 7 X. Xiaoding and J. A. Moulijn, *Energy Fuels*, 1996, **10**, 305–325.
- 8 W. Hou, W. H. Hung, P. Pavaskar, A. Goeppert, M. Aykol and S. B. Cronin, *ACS Catal.*, 2011, **1**, 929–936.
- 9 H. Li, P. H. Opgenorth, D. G. Wernick, S. Rogers, T.-Y. Wu, W. Higashide, P. Malati, Y.-X. Huo, K. M. Cho and J. C. Liao, *Science*, 2012, 335, 1596.
- 10 J. Shi, Y. Jiang, Z. Jiang, X. Wang, X. Wang, S. Zhang, P. Han and C. Yang, *Chem. Soc. Rev.*, 2015, **44**, 5981–6000.
- 11 E. E. Barton, D. M. Rampulla and A. B. Bocarsly, *J. Am. Chem. Soc.*, 2008, **130**, 6342–6344.
- 12 J. Barber, Chem. Soc. Rev., 2009, 38(1), 185-196.
- 13 Y. Amao, N. Shuto, K. Furuno, A. Obata, Y. Fuchino, K. Uemura, T. Kajino, T. Sekito, S. Iwai, Y. Miyamoto and M. Matsuda, *Faraday Discuss.*, 2012, **155**, 289–296.
- 14 Y. Amao, ChemCatChem, 2011, 3(3), 458-474.
- 15 S. Kuwabata, K. Nishida, R. Tsuda, H. Inoue and H. Yoneyama, *J. Electrochem. Soc.*, 1994, **141**(6), 1498–1503.
- 16 Y. Amao and T. Watanabe, *Appl. Catal., B*, 2009, **86**(3–4), 109–113.
- 17 A. Dibenedetto, P. Stufano, W. MacYk, T. Baran, C. Fragale, M. Costa and M. Aresta, *ChemSusChem*, 2012, 5(2), 373–378.
- 18 Y. Zhou, Z. Tian, Z. Zhao, Q. Liu, J. Kou, X. Chen, J. Gao, S. Yan and Z. Zou, ACS Appl. Mater. Interfaces, 2011, 3(9), 3594–3601.
- 19 S. Bensaid, G. Centi, E. Garrone, S. Perathoner and G. Saracco, *ChemSusChem*, 2012, 5, 500–521.
- 20 B. Kumar, M. Llorente, J. Froehlich, T. Dang, A. Sathrum and C. P. Kubiak, *Annu. Rev. Phys. Chem.*, 2012, **63**, 541–569.
- 21 Y. Lu, Z. y. Jiang, S. Wei Xu and H. Wu, *Catal. Today*, 2006, **115**, 263–268.
- 22 J. Luo, A. S. Meyer, R. V. Mateiu and M. Pinelo, *New Biotechnol.*, 2015, **32**, 319–327.
- 23 I. H. Tseng, W. C. Chang and J. C. S. Wu, *Appl. Catal.*, B, 2002, 37, 37–48.
- 24 K. J. Shah and T. Imae, Chem. Eng. J., 2016, 283, 1366-1373.
- 25 R. A. Caruso and J. H. Schattka, *Adv. Mater.*, 2000, 12(24), 1921–1923.
- 26 M. Sakamoto, T. Kamachi, I. Okura, A. Ueno and H. Mihara, *Biopolymers*, 2001, **59**, 103–109.
- 27 S. Cherian and C. C. Wamser, *J. Phys. Chem. B*, 2000, **104**(15), 3624–3629.
- 28 T. Yamazaki and T. Imae, J. Nanosci. Nanotechnol., 2005, 5(7), 1066–1071.
- 29 K. Takahashi, N. Kamimura, S. Hishiyama, H. Hara, D. Kasai, Y. Katayama, M. Fukuda, S. Kajita and E. Masai, *Biodegradation*, 2014, **25**, 735–745.
- 30 Y. Huang, S. Xiong, G. Liu and R. Zhao, Chem. Commun., 2011, 47(29), 8319–8321.
- 31 T. Nash, Biochem. J., 1953, 55(3), 416-421.
- 32 P. W. Wu, C. C. Chang and S. S. Chou, *J. Food Drug Anal.*, 2003, **11**(1), 8–15.
- 33 Y. Y. Zhan, Y. Zhang, Q. M. Li and X. Z. Du, J. Chin. Chem. Soc., 2010, 57(2), 230–235.
- 34 X. Sala, I. Romero, M. Rodriguez, L. Escriche and A. Llobet, *Angew. Chem.*, 2009, 48, 2842–2852.

- Paper
- 35 K. J. Shah, T. Imae, M. Ujihara, S. J. Huang, P. H. Wu and S. B. Liu, *Chem. Eng. J.*, 2017, **312**, 118–125.
- 36 R. Bendi and T. Imae, RSC Adv., 2013, 3, 16279–16282.
- 37 R. Bendi, T. Imae and A. G. Destaye, *Appl. Catal., A*, 2015, 492, 184–189.
- 38 M. A. Kebede, T. Imae, Sabrina, C. M. Wu and K. B. Cheng, *Chem. Eng. J.*, 2017, **311**, 340–347.
- 39 G. Knör, Coord. Chem. Rev., 2015, 304-305, 102-108.
- 40 J. H. Montoya, L. C. Seitz, P. Chakthranont, A. Vojvodic, T. F. Jaramillo and J. K. Nørskov, *Nat. Mater.*, 2017, 16, 70– 81.
- 41 H. M. Woo, Curr. Opin. Biotechnol., 2017, 45, 1-7.
- 42 S. Bhatta, D. Nagassou and J. P. Trelles, *Sol. Energy*, 2017, 142, 253–266.
- 43 J. H. Lee, D. H. Nam, S. H. Lee, J. H. Park, C. B. Park and K. J. Jeong, *J. Ind. Eng. Chem.*, 2016, **33**(4), 28–32.

- 44 T. A. Word and R. W. Larsen, *Arch. Biochem. Biophys.*, 2017, **615**, 10–14.
- 45 C. Hu, Y. Yu and J. Wang, *Chem. Commun.*, 2017, 53, 4173-4186.
- 46 D. H. Nam, S. K. Kuk, H. Choe, S. Lee, J. W. Ko, E. J. Son, E.-G. Choi, Y. H. Kim and C. B. Park, *Green Chem.*, 2016, 5989–5993.
- 47 C. Liu, B. C. Colón, M. Ziesack, P. A. Silver and D. G. Nocera, *Science*, 2016, **352**, 1210–1213.
- 48 Y. Liu and J. Y. Chen, *J. Bioact. Compat. Polym.*, 2016, **31**, 553–567.
- 49 T. Abitbol, A. Rivkin, Y. Cao, Y. Nevo, E. Abraham, T. Ben-Shalom, S. Lapidot and O. Shoseyov, *Curr. Opin. Biotechnol.*, 2016, **39**, 76–88.
- 50 W. Yang, Z. Zhao, K. Wu, R. Huang, T. Liu, H. Jiang, F. Chen and Q. Fu, *J. Mater. Chem. C*, 2017, 5, 3748–3756.