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Photocatalytic Reforming of Lignocelluloses, Glycerol, and Chlorella to Hydrogen

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Additional information is available at the end of the chapter

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Abstract

Bioethanol, biodiesel, and biogas have gained much attention as sustainable energy alternatives to petroleum-based fuels. Bioethanol production is the most typical method to provide liquid fuel. Recently cellulosic materials have been recognized as one of the promising sources for bioethanol, since they are not directly in competition with food sources. However, ethanol concentration is usually too low to separate by distillation at a low-energy cost. Gaseous H₂ is spontaneously isolated without operation to separate. Therefore, H₂ production is an economical approach to biofuels. Photocatalytic H₂ production over a Pt-loaded TiO₂ is initiated by the charge separation. Electrons reduce water to generate H₂, while holes oxidize hydroxide to hydroxyl radicals. Generally, the use of sacrificial agents remarkably accelerates the H₂ production since the hydroxyl radical is consumed by them. This chapter deals with the photocatalytic H₂ production (PR) using sacrificial water-soluble materials derived from lignocelluloses, lipids, and Chlorella. Lignocellulosic Italian ryegrass (2.00 g) was turned into H₂ (78.7 mg) through alkali treatment, hydrolysis, and PR processes. The PR process of glycerol (10.4 g) and methanol (11.3 g), which were by-products in biodiesel synthesis, formed H₂ (3.10 g). Dried Chlorella (10 g) was turned into H₂ (578 mg) by protease hydrolysis and PR.

Keywords: TiO₂, sacrificial agents, lignocelluloses, BDF, Chlorella

1. Introduction

Plants collect sunlight energy through photosynthesis and store it as a variety of polymeric saccharides. Polymeric saccharides are converted into monomeric saccharides, which are then converted into energy in all living organisms. Thus, saccharides are energy-storage substances which are produced from CO₂ and easily converted to energy along with CO₂ emission.

Therefore, saccharides have highly potential resources to produce renewable energy. Bioethanol production from starch of maize, sugarcane, and sugar sorghum is the most typical method to provide renewable liquid fuel [1, 2]. Recently in order to avoid the direct competition with food sources, cellulosic materials have been widely recognized as one of the promising sustainable resources to produce second-generation bioethanol [3]. However, the ethanol concentrations (<5.0 %) were still too low to separate by distillation at a low-energy cost [4]. On the other hand, gaseous H₂ is spontaneously isolated from reaction mixtures without operations to separate. Therefore, H₂ production from saccharides and biomass-derived materials is one of the economical approaches to biofuels [5].

In this chapter, I will show the photocatalytic reforming over titanium dioxide (TiO₂) using saccharides, glycerol, and amino acids, which are derived by hydrolysis of lignocelluloses, lipids, and Chlorella, respectively. This will lead to construct the sustainable energy system alternatives to petroleum-based fuels.

2. Outline of photocatalytic biomass reforming

A general procedure of biomass reforming is started by the production of water-soluble materials from biomass through biological treatment as well as chemical reaction. The resulting water-soluble materials are converted to biofuels such as ethanol, methane, and hydrogen through various catalytic reactions involving methane fermentation and steam reforming. It was demonstrated that the photocatalytic H₂ production from biomass-derived materials had an advantage compared with other thermal catalytic reforming by Shimura and Yoshida in their review in 2011 [6].

Our biomass reforming was performed in aqueous solution through enzymatic and chemical hydrolysis of biomass (lignocelluloses, lipids, and Chlorella) followed by photocatalytic reaction of water-soluble materials (saccharides, glycerol, and amino acids) over TiO₂ under UV-irradiation (**Figure 1**). Saccharides were produced by enzymatic hydrolysis of lignocelluloses using cellulase and xylanase. Glycerol was obtained by transesterification of lipid with methanol. Amino acids were obtained from hydrolysis of Chlorella by protease. These water-soluble materials were served as sacrificial agents for the photocatalytic H₂ production in aqueous solution. Details of each process were described in the following sections.

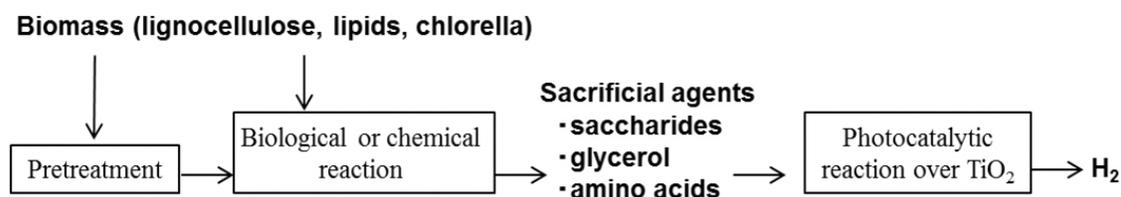


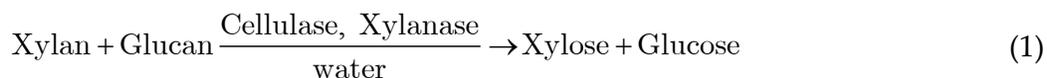
Figure 1. Outline of photocatalytic reforming of biomass.

3. Biological reactions

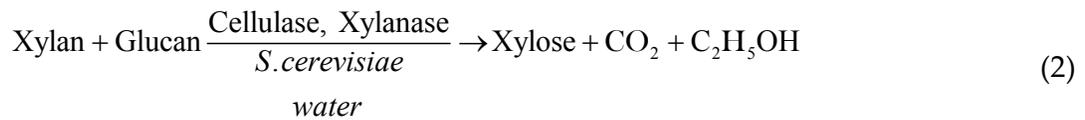
For biological reaction, a cellulase from *Acremonium cellulolyticus* (Acremozyme KM, Kyowa Kasei, Osaka, Japan) [7] was selected among commercially available cellulases. A xylanase from *Trichoderma longibrachiatum* (reesei) (Sumizyme X, Shin Nihon Chemicals, Anjyo, Japan) was selected from commercially available enzymes. Proteins were hydrolyzed by protease (protease A AmanoSD, Amano enzyme, Nagoya) at 50°C in a phosphate buffer (0.1 M, pH 7.6) which was prepared by dissolving Na₂HPO₄ (2.469 g) and NaH₂PO₄ (0.312 g) in 100 mL of water.

The cell suspension of *Saccharomyces cerevisiae* was prepared as follows. *S. cerevisiae* NBRC 2044 was grown at 30°C for 24 h in a basal medium consisting of glucose (20.0 g/L), bactotryptone (1.0 g/L, Difco), yeast extract (1.0 g/L), MaSO₄ (3.0 g/L), and NaHPO₄ (1.0 g/L) at initial pH 5.5 [7].

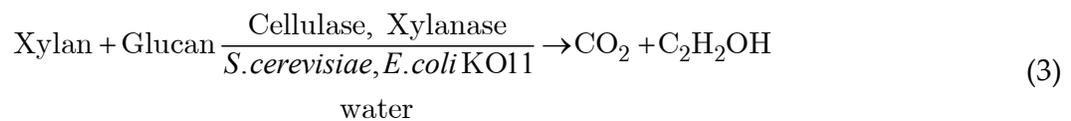
Cellulose and hemicellulose (holocellulose), which were composed of glucan and xylan, were hydrolyzed to glucose and xylose by the enzymatic saccharification (SA, Eq. 1). The powdered and pre-treated lignocellulose (4.0 g) was dispersed in an acetate buffer solution (80 mL, pH 5.0, 0.1 M) which was prepared by mixing 0.808 g acetic acid and 3.05 g sodium acetate in 500 mL of water. Cellulase (200 mg) and xylanase (200 mg) were added to the suspension of lignocellulose. The SA was performed by stirring the solution vigorously with a magnetic stirrer at 45°C for 120 h. After centrifugation of reaction mixture, the supernatant solution involving glucose and xylose was analyzed by HPLC and used as sacrificial agents in the following photocatalytic reaction.



Also, lignocellulose could be turned into ethanol and xylose through simultaneous saccharification and fermentation (SSF, Eq. 2) using cellulase and xylanase as well as *S. cerevisiae* as follows [8]. An acetate buffer solution (10 mL, pH 5.0, 0.1 M) was added to pre-treated lignocelluloses (3.0 g) in the reaction vessel. The reaction vessel was autoclaved at 120°C for 20 min. After cooling, cellulase (180 mg) and xylanase (120 mg) in an acetate buffer solution (8.0 mL) and the cell suspension of *S. cerevisiae* (0.36 mL) were introduced into the reaction vessel. After air was purged with N₂ stream for 15 min, the SSF was performed at 34°C under stirring vigorously with a magnetic stirrer. The evolved CO₂ was collected by a measuring cylinder to monitor the volume of CO₂ gas. The SSF reaction was continued for about 96 h until CO₂ evolution ceased. After unreacted biomass was removed from the reaction mixture by centrifugation, the supernatant solution was analyzed by gas chromatography (GC) and high-performance liquid chromatography (HPLC) to determine the concentrations of ethanol and saccharides, respectively. Ethanol was collected from the SSF solution by evaporation under reduced pressure while the residual xylose was subjected to the photocatalytic reaction.



Another process to convert lignocellulose to ethanol is simultaneous saccharification and co-fermentation (SSCF). A recombinant *Escherichia coli* KO11 which can ferment xylose was used. Glucan and xylan in lignocellulose are turned to ethanol by SSCF using cellulase, xylanase, yeast, and *E. coli* KO11. An example is an SSCF process of the low-moisture anhydrous ammonia (LMAA)-treated Italian ryegrass (Section 6.1), which produced ethanol in 84.6% yield [9]. In this case, it was not necessary to undergo the photocatalytic process.



4. Photocatalytic H₂ production

4.1. Titanium dioxide (TiO₂) as the photocatalyst

TiO₂ is a white powder material which is thermally stable, non-flammable, and no health hazards. Therefore, TiO₂ has been used for many years in industrial and consumer goods, including paints, coated fabrics and textiles, cosmetics, and so on. The photocatalytic H₂ production was performed by use of an anatase-type TiO₂. It has a semi-conductor structure whose band gap is known to be 3.20 eV, which corresponds to 385 nm. Therefore, TiO₂ can be excited by 366 nm-emission from a high-pressure mercury lamp. Irradiation induces charge separation into electrons and holes on the TiO₂ [10]. Electrons (e⁻) reduce water to generate H₂, while holes (h⁺) oxidize hydroxide anions to hydroxyl radicals (**Figure 2**) [11]. In most cases, noble metals (Pt, Pd, and Au) were loaded on TiO₂ to accelerate the reduction of water by electrons. We used a Pt-loaded TiO₂ (Pt/TiO₂) throughout the present investigation.

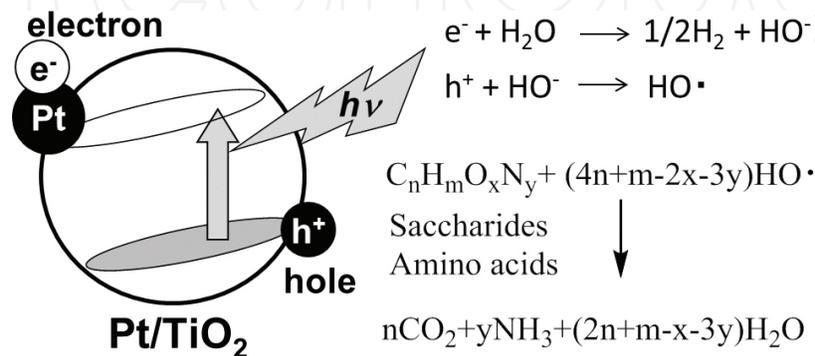


Figure 2. Hydrogen evolution on Pt/TiO₂ under irradiation.

Moreover, it was well known that the use of sacrificial agents remarkably accelerates H_2 production because the hydroxyl radical is consumed by them. Especially, we have elucidated that sacrificial agents with all of the carbon attached heteroatoms (O and N) are superior sacrificial agents because they continued to serve as electron sources until their sacrificial ability was exhausted [12, 13]. Glucose, xylose, glycerol, and glycine meet this requirement. The photocatalytic H_2 production using sacrificial agents is called “sacrificial H_2 production.”

4.2. Preparation of Pt-loaded TiO_2 photocatalyst

For photocatalytic reaction, almost researches have continued to use a P25 (Degussa Co. Ltd, Germany) and a ST01 (Ishihara Sangyo Co. Ltd., Japan). The P25 is prepared through hydrolysis of $TiCl_4$ and composed of 75% of anatase and 25% of rutile, while the ST01 was prepared through hydrolysis of $TiOSO_4$ and composed of 100% of anatase.

The Pt-loaded TiO_2 (Pt/TiO_2) was prepared by the method reported by Kennedy and Datye [14] as follows. An aqueous solution (400 mL) containing TiO_2 (4.0 g, ST01, particle size 7 nm and surface area $300\text{ m}^2\text{g}^{-1}$), K_2PtCl_6 (200 mg), and 2-propanol (3.06 mL) was introduced into a reaction vessel which is illustrated in Section 4.3. After O_2 was purged by N_2 gas, the solution was irradiated by a high-pressure mercury lamp with stirring for 24 h when the gas evolution reached over 100 mL. After the irradiation, water was entirely evaporated. The resulting gray precipitate was moved on a filter and washed with water and then dried and ground to produce Pt/TiO_2 powder. The Pt-content on TiO_2 was optimized to be 2.0 wt% from the photocatalytic H_2 evolution by various Pt-content TiO_2 using glucose as a sacrificial reagent. Identification of Pt/TiO_2 was usually performed by an XRD pattern and TEM image [15]. **Figure 3** shows a TEM image and an X-ray diffraction pattern of a Pt/TiO_2 (2.0 wt% of Pt content).

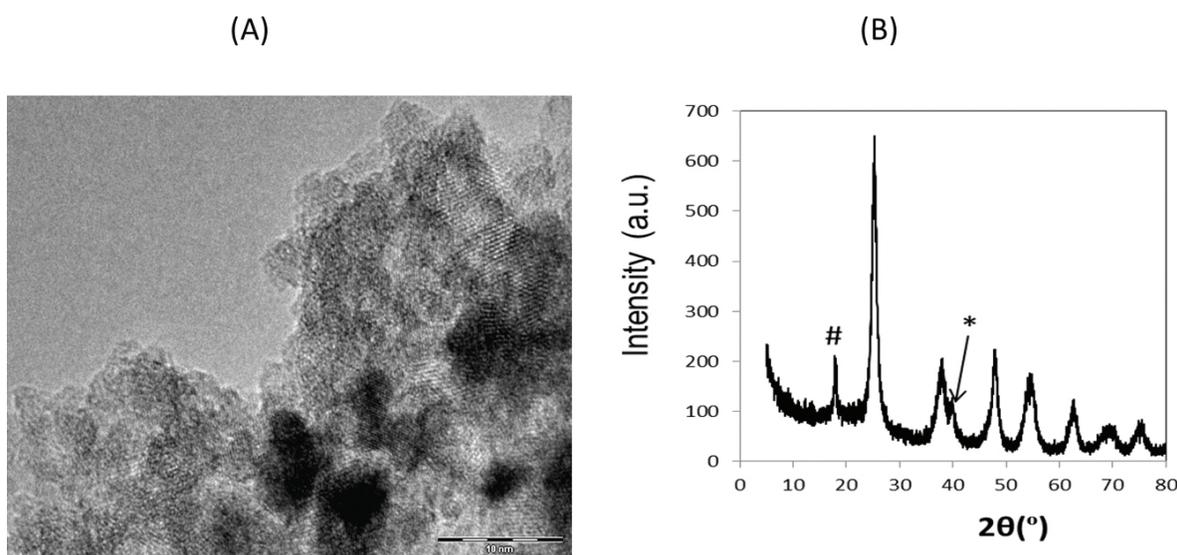


Figure 3. (A) TEM images of Pt/TiO_2 (2.0 wt% of Pt content). (B) X-ray diffraction of a Pt/TiO_2 (2.0 wt% of Pt content). Mark * was the peak for Pt. Mark # was the peak for impurity of Teflon removed from the stirrer chip.

4.3. Experimental method

The photocatalytic H₂ production was performed using a photo-irradiation apparatus (Figure 4). The catalyst (100 mg) and the given amounts of aqueous solution of sacrificial agent were introduced into a reaction vessel. The volume of the reaction solution was adjusted to 150 mL with water. The reaction vessel was connected with a measuring cylinder through a gas-impermeable fluororubber tube to collect the evolved gas. A high-pressure mercury lamp (100 W, UVL-100HA, Riko, Japan) was inserted into the reaction vessel, which was set in a water bath to keep it at a constant temperature (usually 20°C). After O₂ was purged from the reaction vessel by N₂ gas for 15 min, the reaction mixture was irradiated with vigorous stirring using a magnetic stirrer until the gas evolution ceased. The evolved gas was collected by a measuring cylinder to measure the total volume of the evolved gas. The evolved gas (0.5 mL) was obtained using a syringe and was subjected to the quantitative analysis of H₂, N₂, and CO₂, which were performed on a Shimadzu GC-8A equipped with a TCD detector at a temperature raised from 40 to 180°C using a stainless column (3 mmΦ, 6 m) packed with a SHINCARBON ST (Shimadzu). In the absence of sacrificial agents, the H₂ evolution from water was small (<2 mL).

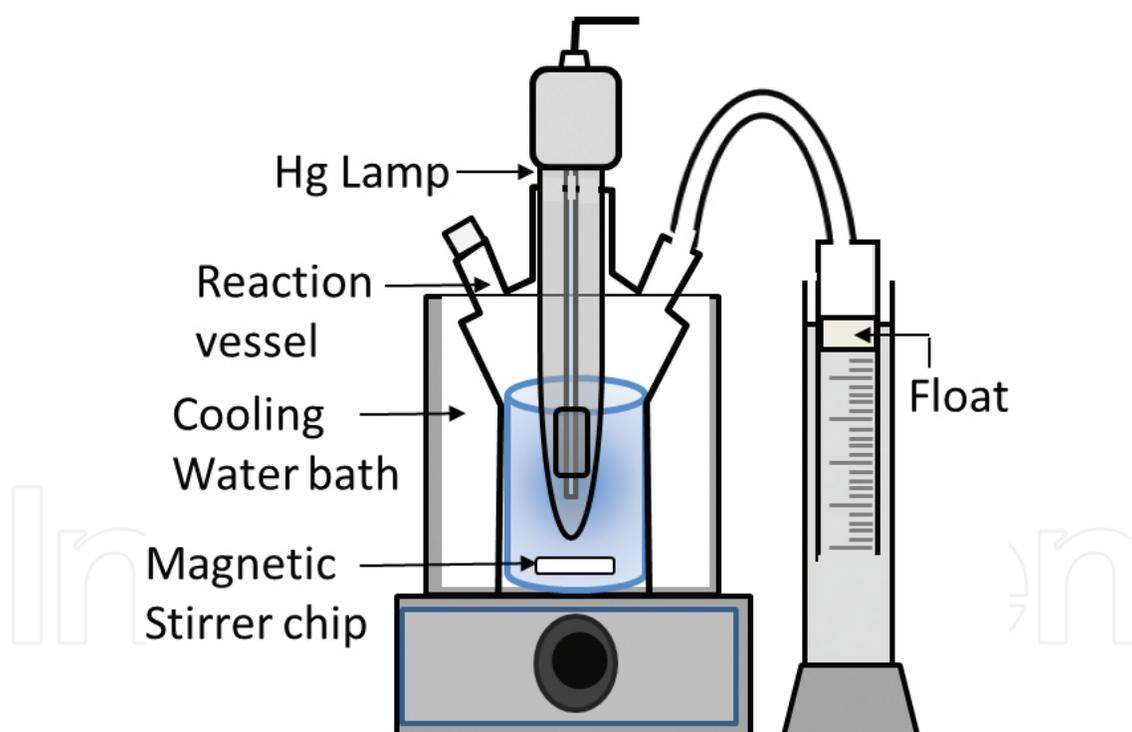
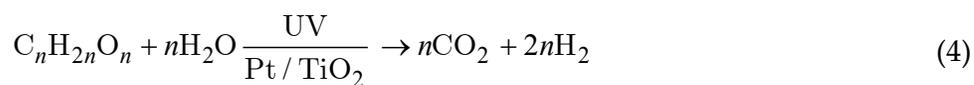


Figure 4. Apparatus for photocatalytic reaction.

4.4. Analysis of photocatalytic reaction

Theoretically, the photocatalytic reaction can convert glucose and xylose to 12 and 10 equivalents of H₂ (Eq. 4). Indeed, the photocatalytic reaction using glucose and xylose produced 11.8 and 10.0 mol of H₂ from 1 mol of glucose [15] and xylose [16], respectively.



I show a method to determine the amounts of H₂ evolved from 1 mol of sacrificial agent. A typical example is the photocatalytic H₂ production using saccharides obtained from enzymatic saccharification of Napier grass. Although the saccharides contained not only xylose but also glucose, the evolved H₂ and CO₂ were plotted against the moles of xylose in a mixture of xylose and glucose, as shown in **Figure 5A**. Gas volumes of H₂ and CO₂ increased with the increase of xylose. However, the molar ratios of H₂ to xylose (H₂/xylose) were not constant to the amount of xylose used. It was speculated that the colored material in the solution and the carboxylic acids formed during the photocatalytic reaction may lower the activity of photocatalyst. Therefore, the H₂/xylose ratio was plotted against the molar ratio of xylose to catalyst (xylose/catalyst), as shown in **Figure 5B**. As the xylose/catalyst ratios decreased, the H₂/xylose ratios increased. The intercept of the plots was equaled to H₂^{max}, which is the limiting mole amount of H₂ produced from one mole of xylose (sacrificial agent) at an infinite amount of catalyst [17]. Thus, the total molar amount of H₂ was calculated by the equation: H₂^{max} × (moles of sacrificial agent).

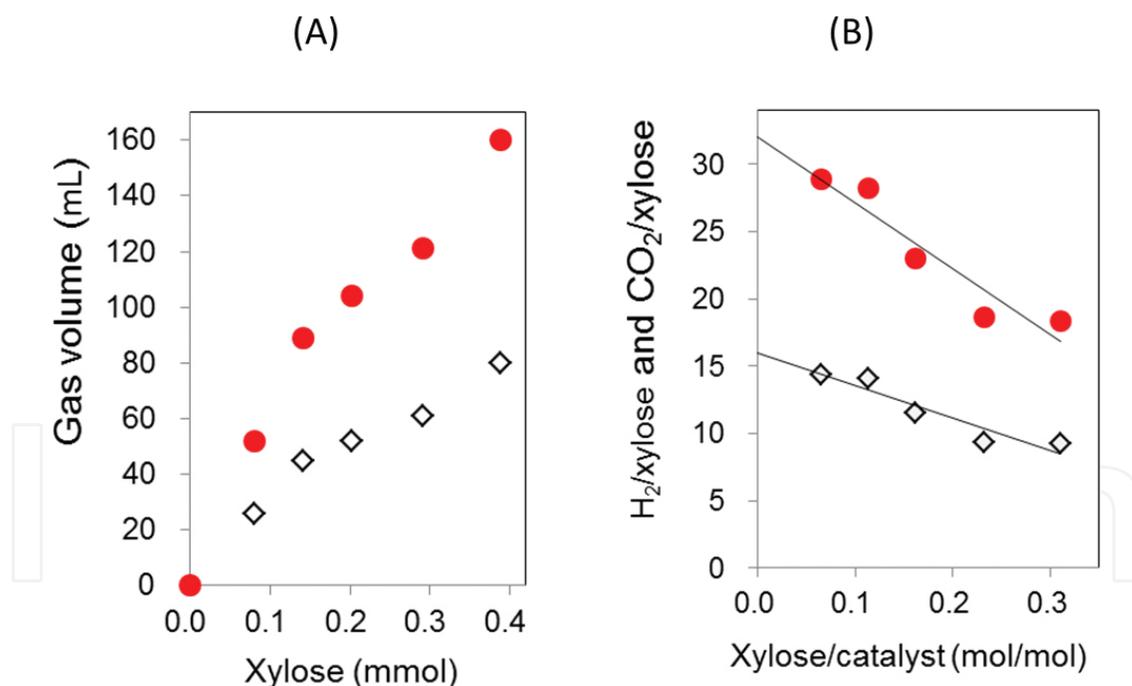


Figure 5. The TiO₂-photocatalytic H₂ production using a mixture of xylose and glucose obtained from the enzymatic saccharification of Napier grass. (A) Dependence of volumes of H₂ (●) and CO₂ (◇) against the mole of xylose. (B) Plots of H₂/xylose (●) and CO₂/xylose (◇) against xylose/catalyst.

Similar plots of CO₂/xylose against the xylose/catalyst were performed, giving the CO₂^{max} values from the intercept of the plots. Other gasses such as methane and CO were not detected in evolved gas.

5. Energy recovery efficiency (E_{ff})

Total energy recovery efficiency (E_{ff}) from biomass to biofuels was calculated using combustion energy: $E_{ff} = 100H_F/H_0$ where H_0 and H_F were the combustion energies of biomass and biofuels, respectively. The combustion energies of sacrificial agents such as glucose, xylose, and glycerol are 2803 [18], 2342 [19], and 1654 kJ/mol [18], respectively. The combustion energies of biofuels such as ethanol and H_2 are 285 and 1367 kJ/mol [18], respectively. In the case of lignocellulose, the H_0 value was combustion energy of glucose and xylose at the complete hydrolysis of glucan and xylan which were determined by the National Renewable Energy Laboratory (NREL) [20].

6. Practical photocatalytic biomass reforming

6.1. Lignocelluloses

Lignocellulosic biomass was composed of cellulose, hemicellulose, lignin, and other components. The components of glucan, xylane, lignin, ash, and others in non-treated lignocelluloses are summarized in **Table 1**. Since the contents of cellulosic components in lignocelluloses were in the range of 41.0–66.5 wt%, only a half of lignocelluloses were utilized for production of H_2 . The method to determine the content of each component was shown as follows.

Lignocelluloses	Contents (wt%)			
	Holocellulose ^a	Lignin	Ash	Others
Italian ryegrass	50.1 (35.1, 15.0)	23.5	12.9	13.5
Napier grass	48.2 (31.3, 16.9)	12.6	13.9	28.7
Bamboo	66.5 (39.5, 26.4)	26.2	1.4	5.9
Rice straw	47.8 (27.9, 19.5)	20.3	17.7	14.2
Silver grass	41.0 (30.8, 10.0)	21.7	4.0	33.3

^aThe values in parenthesis are the contents of glucan and xylan in holocellulose.

Table 1. Components of non-treated lignocelluloses.

Lignocelluloses were cut by a cutter and dried at 70°C for 72 h. The dried matter was powdered by a blender until the powder passed through a sieve with 150 μ m of mesh. The powdered lignocellulose (30 g) was treated with a 1% aqueous solution of NaOH (400 mL) at 95°C for 1 h. The reaction mixture was centrifuged and filtered to isolate the holocellulose (a mixture of cellulose and hemicellulose) as a pale yellow precipitate. The supernatant solution was made acidic (pH 5.0) with a dilute HCl solution to isolate dark brown precipitate which was identified as lignin. The precipitate was collected via centrifugation at 10,000 rpm for 10 min.

The contents of saccharides in holocellulose were analyzed according to the methods published by NREL [20]. Sulfuric acid (72 wt%, 3.0 mL) was added slowly to holocellulose (300 mg) in a reaction vessel and kept at 30°C for 1 h. Water (84 mL) was added to the reaction vessel so that the concentration of sulfuric acid became 4.0 wt%. Acid hydrolysis was performed by autoclaving at 121°C for 1 h in an autoclave. The treated solution was neutralized with CaCO₃ and was centrifuged. The supernatant solution (ca. 87 mL) was concentrated to 30 mL by evaporation. The solution was analyzed by HPLC to determine the amounts of glucose and xylose. The amounts of glucan and xylan were determined from the amounts of glucose and xylose. The ash component in lignocellulose was obtained by the burning of the lignocellulose (2.0 g) in an electric furnace (KBF784N1, Koyo, Nara, Japan) for 2 h at 850°C.

The pre-treatments to promote an enzymatic digestibility of the cellulosic components and to remove the lignin component were usually performed. Alkali (AL) treatment is a popular method to remove lignin from lignocelluloses [21]. A powdered lignocellulose (30 g) was added to a 1% aqueous solution of NaOH (400 mL). The mixture was heated under stirring at 95°C for 1 h. The reaction mixture was subjected to centrifugation at 10,000 rpm for 10 min. The lignin remained in the supernatant solution. The holocellulose, which is a mixture of cellulose and hemicellulose, is isolated as a pale yellow precipitate, which was washed by dispersion in water to remove the contaminated lignin. After the pH adjustment to 7.0, the washed precipitate was collected by centrifugation and dried. Thus, lignin-removed holocellulose was obtained. The AL treatment is effective for saccharification of the lignocellulose with higher lignin contents. However, in the case of lignocelluloses with low lignin content such as Napier grass, the AL treatment retarded the yeast-fermentation rate because AL treatment removed not only lignin but also nutrients to help yeast fermentation [22].

Another useful pretreatment of lignocelluloses is LMAA (low-moisture anhydrous ammonia pretreatment), described as follows [23]. Dry powdered lignocelluloses (100 g, volume 320 mL) were mixed homogeneously with water (100 g) in a flask (1 L). The flask containing wet lignocellulose was evacuated with a pump and then gaseous NH₃ was introduced into the flask repeatedly until the atmosphere inside the flask was entirely replaced with NH₃ gas. The moist powdered lignocellulose was kept under an NH₃ gas atmosphere at room temperature for 28 days. After NH₃ was removed with an evaporator, the treated lignocellulose was washed with water to liberate the brownish aqueous alkali solution of the lignin. This washing operation was continued until the pH became below 7.7. The treated lignocellulose was dried at 60°C. Here, NH₃ served for transformation of the cellulose crystal phase to a highly reactive structure toward enzymatic degradation rather than the removal of lignin [24]. As a special pretreatment method, TiO₂-photocatalytic pretreatment was developed by our group [25].

The photocatalytic reforming was applied to lignocelluloses such as Italian ryegrass [26], Napier grass [26], bamboo [27], rice straw [27], and silver grass [27]. The results are summarized in **Table 2**. The SA→PR method is a process through the enzymatic saccharification (SA) of the pretreated lignocelluloses into glucose and xylose which were then used as sacrificial agents for the photocatalytic H₂ production over Pt/TiO₂ (PR). For example, the dried Italian ryegrass (2.00 g) was subjected to the AL treatment to give the AL-treated Italian ryegrass (1.00 g) which was turned into 554 mg of glucose and 193 mg of xylose by SA. The

SA of xylan was more inefficient than that of glucan. Glucose and xylose were turned into H_2 (78.7 mg) by PR. As a result, the total energy recovery efficiency (E_{ff}) from AL-treated Italian ryegrass to H_2 was calculated to be 71.9% (Figure 6). In the case of Napier grass, dried Napier grass (2.075 g) was subjected to the AL treatment to give the AL-treated Napier grass (1.00g) which was turned into 487 mg of glucose and 197 mg of xylose by SA. The PR of glucose and xylose gave 84.0 mg of H_2 , which corresponded to 77.0% of E_{ff} .

Biomass	Process ^a	Biofuels								
		PT ^b	W_G^c (mg)	W_X^c (mg)	H_0^d (kJ)	EtOH (mg)	H_2 (mg)	H_f^e (kJ)	E_{ff}^f (%)	
Italian ryegrass	LMAA		480	206	11.96	SSCF	333	0	9.90	82.7
Italian ryegrass	AL		700	300	15.58	SA→PR	0	78.7	11.20	71.9
Italian ryegrass	LMAA		480	206	11.96	SSF→PR	250	17.3	9.89	82.7
Napier grass	AL		651	350	15.60	SA→PR	0	84.0	12.0	77.0
Napier grass	LMAA		398	214	10.68	SSF→PR	177	21.0	8.25	77.2
Bamboo	AL		594	396	17.30	SSF→PR	213	44.5	12.68	73.4
Rice straw	AL		756	238	17.30	SSF→PR	364	34.8	15.76	91.1
Silver grass	AL		749	253	17.28	SSF→PR	323	35.7	14.68	85.0

^aSSF = simultaneous saccharification and fermentation using cellulase and yeast. SA = enzymatic saccharification. PR = photocatalytic H_2 production over Pt/TiO₂. SSCF= Simultaneous saccharification and co-fermentation using cellulase, yeast, and recombinant *E. coli* KO11. Referred from reference [9].

^bPT, pretreatment; LMAA, low moisture anhydrous ammonia pretreatment; AL, alkali pretreatment.

^c W_G and W_X were the amounts of glucan (G) and xylan (X) per 1 g of the pretreated lignocellulose.

^dThe total combustion energies (H_0) of xylose and glucose theoretically derived from 1.0 g of the pretreated lignocelluloses were calculated according to the following equation: $H_0 = 2803 \times W_G/162 + 2342 \times W_X/132$.

^eTotal combustion energy (H_f) of biofuels (ethanol and hydrogen).

^fEnergy recovery efficiency (E_{ff}) = $100 \times H_f/H_0$.

Table 2. Biofuel production from lignocelluloses.

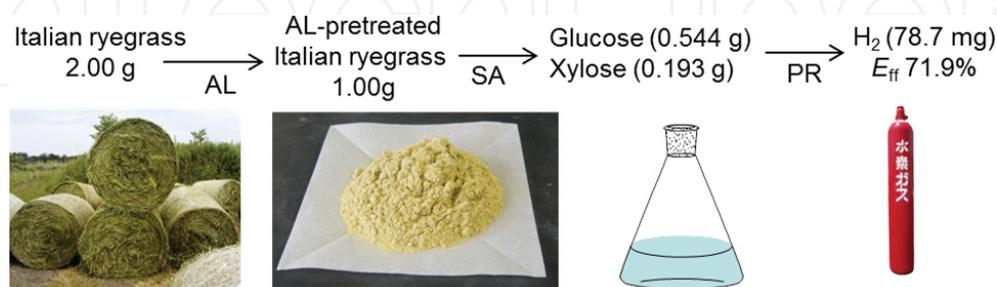


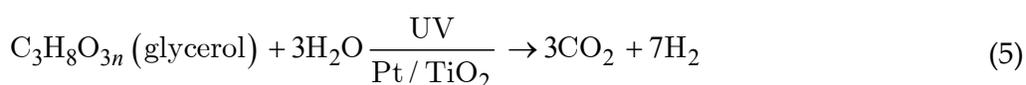
Figure 6. AL→SA→PR process of Italian ryegrass.

In the case of the SSF→PR method, the LMAA treatment of the dried Italian ryegrass (1.458 g) gave the LMAA-treated Italian ryegrass (1.0 g) which was turned into ethanol (250 mg), xylose

(121 mg), and glucose (19 mg) by SSF process. Ethanol was removed from SSF solution, whereas the residual xylose and glucose were converted to H₂ (17.3 mg) by PR. The E_{ff} value of H₂ combined with ethanol was 82.7% from the LMAA-treated Italian ryegrass. We have reported the ethanol production through an SSCF process of Italian ryegrass [9]. The E_{ff} value was 82.7%. These E_{ff} values showed similar values. In the cases of Napier grass, the LMAA treatment of the dried Napier grass (1.637 g) gave the LMAA-treated Napier grass (1.0 g) which was turned into ethanol (177 mg), xylose (167 mg), and glucose (13 mg) by SSF process. After ethanol was removed from SSF solution, the residual xylose and glucose were converted to H₂ (21.0 mg) by PR. The E_{ff} value of H₂ combined with ethanol was 77.2% from the LMAA-treated Napier grass. In the cases of bamboo, rice straw, and silver grass, the AL treatment of bamboo (1.656 g), rice straw (2.092), and silver grass (2.439 g) produced the AL-treated lignocelluloses (1.00 g). They were turned into ethanol and H₂ by the SSF→PR process with E_{ff} of over 73.4%.

6.2. Glycerol

Biodiesel (BDF) is one of new sustainable energy alternatives to petroleum-based fuels. BDF market has significantly increased in Europe to adhere energy and climate policies [28]. BDF (methyl alkanoate) is produced by transesterification of vegetable oil or animal fats with methanol under basic conditions [29]. However, glycerol as co-production and unreacted methanol was not utilized and went to waste. Glycerol has a potential to produce H₂ in maximum theoretical yield of seven equivalents (Eq. 5). Also methanol can produce three equivalents of H₂. Hydrogen transformation of glycerol and unreacted methanol isolated from the BDF synthesis was performed by sacrificial H₂ production over a Pt/TiO₂ [30].



As starting material, we used vegetable oil which was mainly composed of oleic acid (C₁₇H₃₃CO₂H) triglyceride. The average molecular weight of vegetable oil was thought to be 884 g/mol. Vegetable oil (150 mL, 136.5 g, 0.154 mol) was set in a reaction vessel. Methanol (30 mL, 23.8 g, 0.743 mol) was mixed with NaOH (0.485g, 0.012 mol). About half of the mixture of methanol and NaOH was poured into a reaction vessel and then kept at 61°C for 1 h. Moreover, the remaining mixture of methanol and NaOH was added into the reaction vessel and the reaction mixture was kept at 61°C for another 1 h. After cooling, the reaction mixtures were separated into a lower layer and an upper layer. The procedure of the follow-up process is shown in **Figure 7**. The lower layer (GL layer) contained glycerol (GL, 0.113 mol) and methanol (0.214 mol). The upper layer (BDF layer) was washed with water (300 mL) to give BDF (114.5 g, 0.387 mol) and the aqueous washing solution which contained 0.137 mol of methanol. The total recovery yield of unreacted methanol was 47.5%. The yields of GL and BDF were 73.3 and 83.7%, respectively.

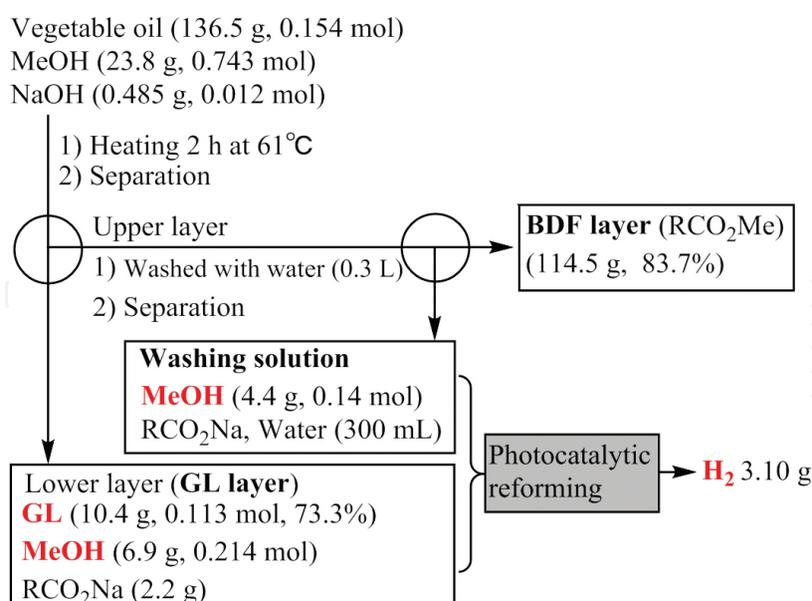


Figure 7. Outline for preparation of BDF and the follow-up process.

The photocatalytic reaction was performed by irradiation of aqueous solution containing Pt/TiO₂ powder (100 mg, 1.25 mmol) and GL layer, which was added to the reaction vessel so that the amounts of GL became 0.25, 0.50, 0.75, 1.00, and 1.25 mmol. The limiting mole amount of H₂ (H_2^{\max}) per 1 mol of GL was obtained from the plots of the H₂/GL against the GL/catalyst. Similarly the photocatalytic reaction was performed for the washing solution, which contained methanol. Using H_2^{\max} values, it was calculated that 2.82 and 0.28 g of H₂ was obtained from the GL layer and washing solution, respectively. The E_{ff} value of H₂ was determined to be 100.8% using H_F of H₂ (444 kJ) and the sum of combustion energy of glycerol ($H_0 = 187$ kJ) and unreacted methanol ($H_0 = 255$ kJ).

6.3. Chlorella

Chlorella is single-cell green algae with 2–10 μm diameter and multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals [31]. Chlorella is mostly composed of proteins (45%), lipids (20%), saccharides (20%), and minerals (10%). Thus, the content of saccharides is low, suggesting that ethanol production is inefficient.

We examined the photocatalytic H₂ production from Chlorella [32]. The frozen Chlorella was thawed and dried in a drying machine and then ground. Gas evolution did not occur from the non-enzymatic-treated solution, which was prepared by magnetic stirring of the Chlorella powder (10 g) in a phosphate buffer (60 mL) for 48 h at 50°C. Therefore, the enzymatic hydrolysis of Chlorella powder (10 g) was performed using protease (1.0 g) in a phosphate buffer (0.1 M, pH 7.6, 60 mL) under stirring at 50°C for 48 h to give the enzymatic hydrolyzed solution. The solution was subjected to centrifugation to remove the precipitate. The supernatant solution (EH solution) was collected. The EH solution was subjected to freezing-drying in order to weigh the water-soluble components in the EH solution. It was determined to be 117 g/L. Since the weight of the solid was 167 g/L before hydrolysis, more than 70% of the solid

was hydrolyzed into water-soluble components. The EH solution was composed of 98.0 g/L of amino acids and 18.3 g/L of glucose which were determined by colorimetric analysis using ninhydrin and by HPLC analysis, respectively.

The photocatalytic H₂ production was performed using the EH solution (0.10 – 0.50 mL) over a Pt/TiO₂ (100 mg) in 150 mL of water. The limiting volume of H₂ per 1 mL of the EH solution (H_2^{\max}) was determined to be 119 mL/mL from the plots of the H₂/(EH solution) against the (EH solution)/catalyst. We successfully produced 579 mg of H₂ from 10.0 g of dry Chlorella (**Figure 8**). This yield is higher than 394 mg for the H₂ production through AL treatment, saccharification, and photocatalytic H₂ production from non-treated Italian ryegrass (10.0 g) [25, 27]. Thus, the photocatalytic reforming is applicable to not only saccharides but also amino acids.

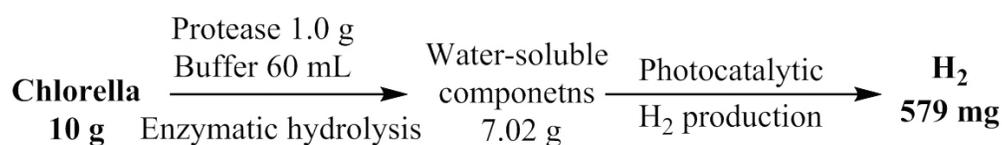


Figure 8. Mass balance for the H₂ production from Chlorella.

Chlorella includes colored materials such as chlorophyll which may disturb the light absorption by the catalyst. Therefore, dried Chlorella (20 g) was subjected to refluxing in ethanol (100 mL) for 6 h to remove the colored materials. Almost all amount of colored materials remained in the ethanol solution. However, the decolorization did not affect the amount of H₂ but could shorten the irradiation time.

7. Conclusions

We examined photocatalytic H₂ production using sacrificial saccharides, glycerol, and amino acid derived from lignocelluloses, lipids, and Chlorella. As a conclusion, the photocatalytic reforming of biomass has the following features:

1. The photocatalytic reforming can be performed in aqueous solution as well as in biological treatment.
2. Gaseous H₂ can spontaneously isolate from aqueous reaction mixtures without operations to be separated.
3. Although it is not easy to produce ethanol from saccharides other than glucose since they are not fermented by yeast (*S. cerevisiae*), sacrificial hydrogen production is applicable to a variety of water-soluble materials.
4. The photocatalytic reforming of biomass is one of the promising approaches because biomass is abundant, clean, and renewable. If sacrificial H₂ evolution is practically accomplished by the use of solar radiation, this method will provide new ways to produce sustainable energy.

In this chapter, biohydrogen production was discussed from the viewpoints of feedstock and methodology to transform biomass to fuels. This will help life cycle assessment (LCA) to evaluate CO₂ emission during cultivation, transportation, and manufacturing, as performed for bioethanol from cellulose [33].

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