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New Approach to Fuelization of Herbaceous Lignocelluloses through Simultaneous Saccharification and Fermentation Followed by Photocatalytic Reforming

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Received: 1 May 2014; in revised form: 13 June 2014 / Accepted: 19 June 2014 /

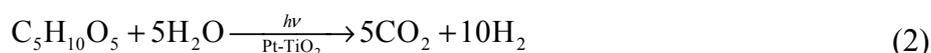
Published: 26 June 2014

Abstract: Bio-fuelization of herbaceous lignocelluloses through a simultaneous saccharification and fermentation process (SSF) and photocatalytic reforming (photo-Reform) was examined. The SSF of the alkali-pretreated bamboo, rice straw, and silvergrass was performed in an acetate buffer (pH 5.0) using cellulase, xylanase, and *Saccharomyces cerevisiae* at 34 °C. Ethanol was produced in 63%–85% yields, while xylose was produced in 74%–97% yields without being fermented because xylose cannot be fermented by *S. cerevisiae*. After the removal of ethanol from the aqueous SSF solution, the SSF solution was subjected to a photo-Reform step where xylose was transformed into hydrogen by a photocatalytic reaction using Pt-loaded TiO₂ (2 wt % of Pt content) under irradiation by a high pressure mercury lamp. The photo-Reform process produced hydrogen in nearly a yield of ten theoretical equivalents to xylose. Total energy was recovered as ethanol and hydrogen whose combustion energy was 73.4%–91.1% of that of the alkali-pretreated lignocelluloses (holocellulose).

Keywords: bamboo; rice straw; silvergrass; cellulase; xylanase; simultaneous saccharification and fermentation (SSF); hydrogen-evolution; Pt-loaded TiO₂

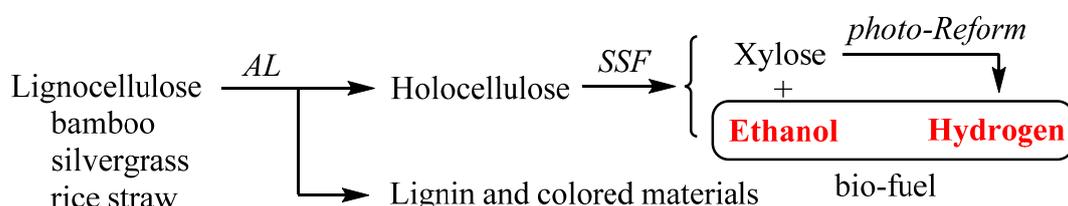
1. Introduction

Ethanol production from biomass has been receiving a great amount of interest from the viewpoint of being a renewable energy alternative to petroleum-based fuels [1]. Second generation bioethanol production from lignocellulosic biomass has been recognized as one of the promising approaches, since the lignocelluloses are not directly in competition with food sources [2]. Usual ethanol production from lignocellulose is conveniently achieved by simultaneous saccharification and fermentation (SSF) using *Saccharomyces cerevisiae* and hydrolytic enzymes [3,4]. However, the ethanol yield is low compared with the first generation bioethanol produced from starches which are composed of glucose units (Equation (1)), because of the high content of hemicellulose composed of xylose units, which are not utilized by *S. cerevisiae*. Therefore fermentation of xylose has been performed using recombinant species of *Escherichia coli* [5–8] and *S. cerevisiae* [9–12]. In order to develop a more convenient methodology to utilize xylose, we intend to develop photocatalytic reforming of xylose to hydrogen (Equation (2)) using a Pt-loaded titanium oxide (Pt-TiO₂) [13]:



The photocatalytic hydrogen evolution from H₂O by the Pt-TiO₂ is initiated by the charge-separation on TiO₂ under photoexcitation [14]. The electrons reduce water to generate H₂ on Pt while holes oxidize hydroxide to hydroxyl radical. It is well known that the use of electron-donating sacrificial agents remarkably accelerates TiO₂-photocatalyzed hydrogen evolution since the hydroxyl radical is consumed by the sacrificial agents [15]. Recently, we have found that sacrificial agents with all of the carbon attached oxygen atoms such as saccharides (e.g., glucose and xylose) and polyalcohols (e.g., 1,2-ethanediol, glycerol, and arabitol) serve as an electron source until their sacrificial ability was exhausted in the TiO₂-photocatalytic hydrogen evolution [16]. Therefore, our attention has been focused on the photocatalytic reforming (photo-Reform) of biomasses using Pt-TiO₂ photocatalyst. Here we examined a new approach to fuelization of bamboo, rice straw, and silvergrass through SSF followed by photo-Reform (Scheme 1).

Scheme 1. Conversion of lignocelluloses to hydrogen through SSF followed by photo-Reform. Operation: *AL*: alkali-pretreatment; *SSF*: simultaneous saccharification and fermentation; *photo-Reform*: photocatalytic reforming using a Pt-TiO₂ catalyst.



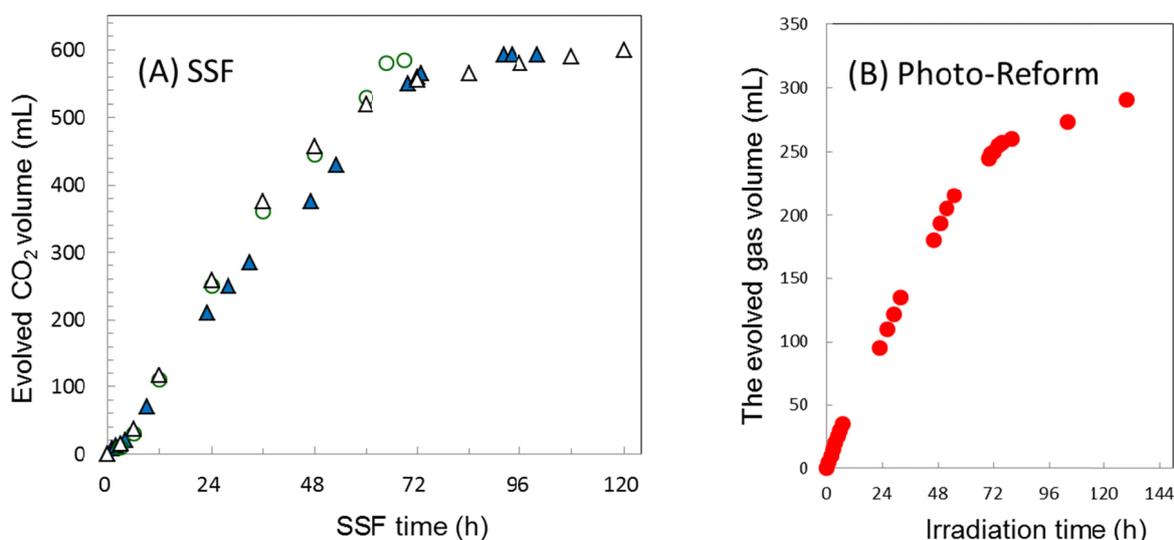
2. Results and Discussion

2.1. SSF Process

In order to remove colored materials such as lignin and chlorophyll, which disturb the light-absorption of the Pt-TiO₂, the powdered lignocellulose was treated with a 1% aqueous solution of NaOH at 95 °C for 1 h. The colored materials were dissolved in the aqueous NaOH solution. The holocellulose (a mixture of cellulose and hemicellulose) was isolated as a pale yellow precipitate by centrifugation. After the pH-adjustment to 7.0, the washed holocellulose was collected by centrifugation and dried.

The alkali-pretreated lignocellulose (holocellulose) was converted into ethanol and xylose by SSF which was performed in an acetate buffer solution at 34 °C using cellulase, xylanase, and the suspension solution of *S. cerevisiae*. The progress of SSF was monitored by the volume of CO₂ and continued for 59–144 h until the CO₂ evolution ceased. The time-conversion of the CO₂-evolution is shown in Figure 1A.

Figure 1. (A) Time-conversion plots of the CO₂ evolution in SSF reaction of alkali-pretreated bamboo (▲), rice straw (△), and silvergrass (○); (B) Typical time-conversion plots of the total gas volume evolved from photo-Reform of xylose which was obtained from SSF of bamboo.



Holocellulose was hydrolyzed to glucose and xylose by a mixture of cellulase and xylanase. We have recently found that the use of xylanase in addition to cellulase accelerates the SSF process of lignocelluloses [17]. Glucose was smoothly fermented to ethanol by *S. cerevisiae* according to Equation (1). However, xylose remained without being fermented by *S. cerevisiae*. Table 1 summarizes the yields of ethanol and xylose in SSF, which were expressed as average of experiments performed three times. Thus, ethanol and xylose were produced in 63%–85% and 74%–97% yields based on the amounts of glucan and xylan in holocellulose listed in Table 1, respectively. Glucose was consumed completely to the point it was not detected in the SSF solution at all.

Table 1. Production of ethanol and xylose through SSF of lignocelluloses.

Lignocellulose	Components (wt %)			SSF process ^(a)		
	Holocellulose ^(b) (glucan and xylan)	Lignin	Others	Time (h)	Ethanol (g) (yield (%))	Xylose (g) ^(c) (yield (%))
Bamboo	60.4 (35.9, 23.9)	17.1	22.5	72	1.29 ± 0.1 (63)	2.02 ± 0.2 (74)
Rice straw	39.0 (29.5, 9.3)	21.3	39.7	144	1.42 ± 0.0 (85)	1.01 ± 0.1 (96)
Silvergrass	37.5 (28.1, 9.5)	23.0	39.5	59	1.21 ± 0.1 (76)	1.00 ± 0.4 (97)

^(a) The SSF of the holocellulose was performed in a degassed acetate buffer solution (pH 5.0, 60 mL) at 34 °C using the cell suspension of *S. cerevisiae* (1.2 mL), cellulase (0.60 g), and xylanase (0.40 g). The amounts of holocellulose were set to 6.04 g, 3.90 g, and 3.75 g for bamboo, rice straw, and silvergrass respectively. Yields were based on the amounts of glucan and xylan in holocellulose; ^(b) Holocellulose was obtained by the pretreatment of lignocelluloses (50 g) with a 1% aqueous solution of NaOH (600 mL) at 95 °C for 1 h. The components of glucan and xylan were analyzed according to NREL method; ^(c) The weight of xylose was represented as W_x .

2.2. Photo-Reform of Xylose

2.2.1. Determination of Limiting Mole Amount of Hydrogen Evolved from Photo-Reform

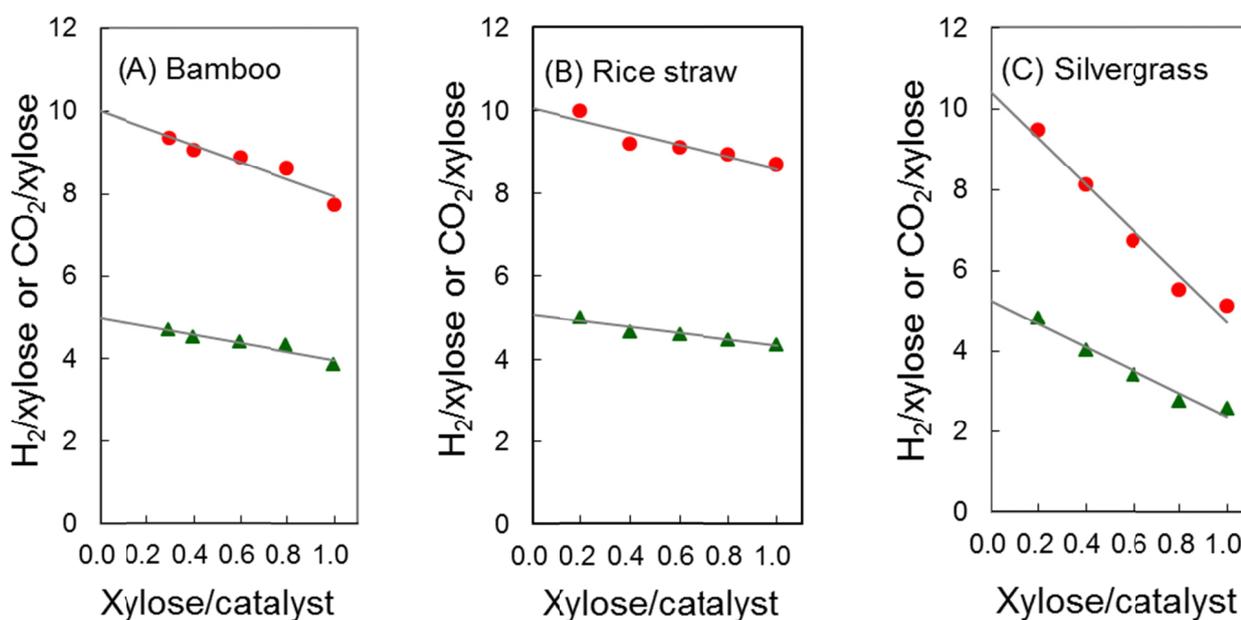
Ethanol was recovered from the SSF solution by distillation under reduced pressure. The residual xylose in the SSF solution was subjected to the photo-Reform step. Pt-TiO₂ (100 mg, 1.25 mmol, 2 wt % of Pt) was introduced to the reaction vessel which was attached to the measuring cylinder. The SSF solution was added in reaction vessel so that the amounts of xylose became 0.25 (0.35), 0.50, 0.75, 1.00, and 1.25 mmol, and then the volume of the solution was adjusted to 150 mL by adding water. After the oxygen was purged from the suspension by bubbling it with N₂ gas, the irradiation was performed by a high-pressure mercury lamp under vigorous stirring with magnetic stirrer until the gas evolution ceased. Typical time conversion of the evolved gas is shown in Figure 1B.

Table 2 lists that the evolved gas volumes. The evolved gas increased with the increase of xylose. However, the molar ratios of H₂ and CO₂ to xylose (H₂/xylose and CO₂/xylose) were not proportional to the amount of xylose used. Therefore, the H₂/xylose values were plotted against the molar ratio of xylose to catalyst (xylose/catalyst), as shown in Figure 2. As the xylose/catalyst values decreased, the H₂/xylose values increased. The intercept of the plots represents H_2^{\max} which is the limiting mole amount of H₂ obtained from one mole of xylose at an infinite amount of catalyst. The H_2^{\max} values were nearly equal to the theoretical value (10.0) shown in Equation (2). The slopes of the plots were changed by the use of lignocellulose. It is possible that they would be affected by the amounts of the materials to lower catalytic activity. Also the limiting mole amount of CO₂ (CO_2^{\max}) were nearly equal to the theoretical values (5.0). Moreover, it was confirmed that the H₂ evolution from water was small (2 mL) in the absence of xylose. Other gases such as CH₄ and CO were not observed in the evolved gas.

Table 2. Photo-Reform using the xylose (**1**) derived from SSF ^(a).

1 ^(b) (H ₂ (g))	Run No.	1 (mg) (1/catalyst)	Irradn. Time (h) ^(c)	Volume (mL)			Molar Ratio		H_2^{\max} ^(e) (CO ₂ ^{max})	H ₂ (g) ^(f) (yield (%))
				Total ^(d)	H ₂	CO ₂	H ₂ /1	CO ₂ /1		
From bamboo (0.362)	1	53 (0.3)	52	110	73	37	9.35	4.68	10.0 (5.0)	0.269 (74)
	2	75 (0.4)	58	152	101	51	9.05	4.52		
	3	113 (0.6)	100	166	149	74	8.87	4.40		
	4	150 (0.8)	147	230	193	97	8.62	4.33		
	5	188 (1.0)	124	270	216	108	7.71	3.86		
From rice straw (0.141)	6	38 (0.2)	69	84	56	28	10.00	5.00	10.1 (5.1)	0.136 (96)
	7	75 (0.4)	74	155	103	52	9.20	4.64		
	8	113 (0.6)	87	230	153	77	9.11	4.58		
	9	150 (0.8)	102	300	200	100	8.93	4.46		
	10	188 (1.0)	130	355	243	122	8.68	4.36		
From silvergrass (0.139)	11	38 (0.2)	49	80	53	27	9.46	4.82	10.1 (5.0)	0.134 (97)
	12	75 (0.4)	58	136	91	45	8.13	4.02		
	13	113 (0.6)	70	170	113	57	6.73	3.39		
	14	150 (0.8)	106	185	123	62	5.49	2.77		
	15	188 (1.0)	155	215	143	72	5.11	2.57		

^(a) Irradiation was performed for an aqueous solution (150 mL) containing xylose (**1**) and Pt-TiO₂ (100 mg, 1.25 mmol); ^(b) The values in the blanket were the theoretical weight of hydrogen (W_0) obtained from 10 g of non-pretreated lignocelluloses; ^(c) Irradiation time until CO₂ evolution ceased; ^(d) Total volume of the evolved gas; ^(e) The H_2^{\max} and CO_2^{\max} were obtained from Figure 2; ^(f) The weight of H₂ (W_H) was calculated by the multiplication of the H_2^{\max} by the W_X , which was the weight of xylose obtained from the SSF listed in Table 1: $W_H = (2/150) H_2^{\max} \times W_X$. The yields of H₂ = $100 \times W_H/W_0$.

Figure 2. Plots of H₂/xylose (●) and CO₂/xylose (▲) against xylose/catalyst: (A) bamboo; (B) rice straw; and (C) silvergrass.

The weight of H_2 (W_H) was calculated by the multiplication of the H_2^{\max} by the W_X which was the weight of xylose obtained from the SSF listed in Table 1: $W_H = (2/150) H_2^{\max} \times W_X$. Thus, the amounts of H_2 derived from 10 g of non-pretreated bamboo, rice straw, and silvergrass were considered to be 0.269 g, 0.136 g, and 0.134 g, which corresponded to 74%, 96%, and 97% yield, respectively. Since the hydrogen evolution proceeded quantitatively, these yields for hydrogen evolution were same yields as the xylose yields listed in Table 1.

2.2.2. Reuse of Photocatalyst

The Pt-TiO₂ was used repeatedly in the photo-Reform in order to check the activity of the photocatalyst. After photo-Reform was performed on Pt-TiO₂ (1.25 mmol) using xylose (1.25 mmol), more xylose (1.25 mmol) was added in the reaction vessel and irradiated until gas evolution ceased to measure the H_2 /xylose values. This operation was repeated eleven times. Figure 3 shows the plots of H_2 /xylose against number of repeated cycles using xylose which was obtained from SSF of bamboo (Table 2 run 5). As number of cycle increased, the H_2 /xylose gradually decreased from 7.71 to 4.90, and the irradiation time increased from 124 h to 174 h. The structure of the Pt-TiO₂ was analyzed by an X-ray diffractometer (Shimadzu XRD 7000). In Figure 4, X-ray diffraction pattern of Pt-TiO₂ catalyst after being used eleven times was compared with that before use. This shows that an anatase structure of TiO₂ was kept even after being used in eleven times. Therefore, the materials to lower catalytic activity would be accumulated in the reaction vessel. It is well known that the carboxylic acid can be strongly adsorbed on TiO₂. Therefore, it is suggested that the adsorption of carboxylic acid derived from xylose and impurity in SSF solution on TiO₂ lowered the photocatalytic activity of Pt-TiO₂.

Figure 3. Dependence of the evolved H_2 amounts (●) and irradiation time (+) on number of cycles in photo-Reform of xylose obtained from the SSF of bamboo.

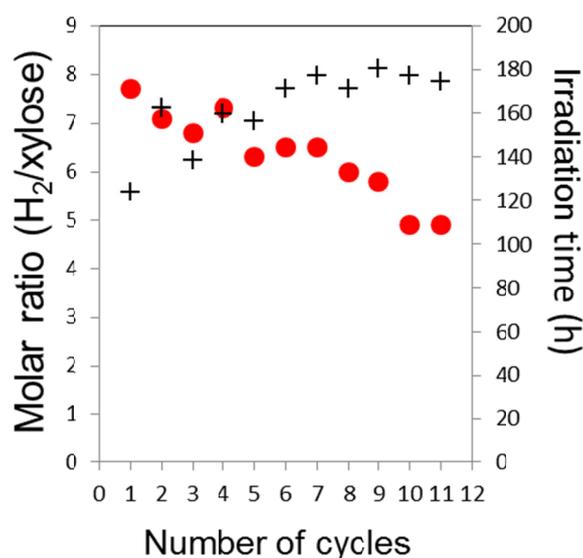
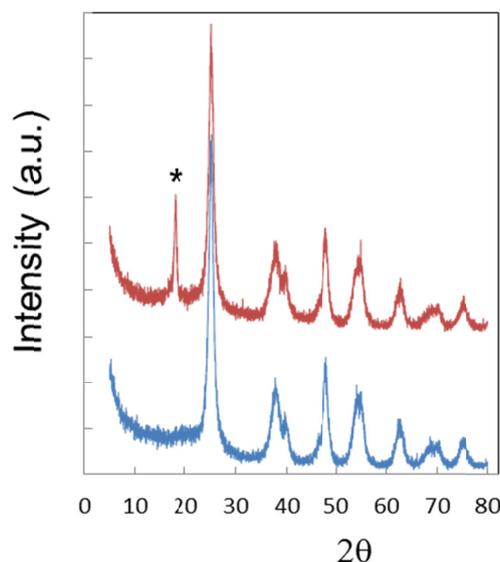


Figure 4. X-ray diffraction of Pt-loaded TiO₂ of before use (blue line) and after use (red line). The symbol * indicates the peak of Teflon removed from the magnate chip during magnetic stirring.



2.3. Evaluation by Combustion Energy

Total combustion energy (H_F) of ethanol and hydrogen [18] produced by SSF and photo-Reform was calculated, as shown in Table 3. Energy recovery efficiency (E_{ff}) was calculated by the ratio of H_F to total combustion energy (H_0) of xylose and glucose produced theoretically from 10 g of the lignocelluloses: $E_{ff} = 100 \times H_F/H_0$. The E_{ff} were considered to be 73.4%–91.1%. Since one mole of xylose has the potential to produce ten equivalent of hydrogen (Equation (2)), photo-Reform of xylose can theoretically produce 2850 kJ of combustion energy of hydrogen which was larger than combustion energy of one mole of xylose (2336 kJ). Therefore, the energy yields were much higher than the product yields.

Table 3. Comparison between lignocellulose and biofuels in the combustion energy.

Lignocellulose		Biofuels		H_F (kJ) ^(c)	E_{ff} (%) ^(d)
H_0 (kJ) (W_G , W_X) ^(a)		H (kJ) ^(b) (weight (g))			
		C ₂ H ₅ OH	H ₂		
Bamboo	104.5 (3.99, 2.72)	38.3 (1.29)	38.3 (0.269)	76.6	73.4
Rice straw	67.6 (3.28, 1.06)	42.2 (1.42)	19.4 (0.136)	61.6	91.1
Silvergrass	64.8 (3.12, 1.04)	36.0 (1.21)	19.1 (0.134)	55.1	85.0

^(a) The total combustion energies (H_0) of xylose and glucose contained in 10 g of the lignocelluloses were calculated according to the following equation: $H_0 = 2803 \times W_G/180 + 2342 \times W_X/150$ where W_G and W_X were the weights of glucose and xylose in g. The combustion energies for glucose and xylose were 2803 kJ mol⁻¹ and 2342 kJ mol⁻¹, respectively; ^(b) Combustion energy (H); ^(c) Total combustion energy (H_F) of ethanol and hydrogen. The combustion energies of ethanol and hydrogen were 1367 kJ mol⁻¹ and 285 kJ mol⁻¹, respectively; ^(d) Energy recovery efficiency (E_{ff}) = $100 \times H_F/H_0$.

3. Materials and Methods

3.1. Chemical Components of Lignocellulose

First, lignocelluloses were cut, dried, and made into a powder by a blender until the powder passed through a sieve with 150 μm mesh. The powdered lignocellulose (30 g) was treated with a 1% aqueous solution of NaOH (400 mL) at 95 °C for 1 h. The holocellulose was isolated as a pale yellow precipitate from the treated mixture by centrifugation at 10,000 rpm for 10 min and filtration. The supernatant solution was neutralized to pH 5.0 by a dilute HCl solution. The resulting dark brown precipitate, which was identified as lignin, was collected by centrifugation at 10,000 rpm for 10 min. Saccharides in the holocellulose were determined according to the methods published by the National Renewable Energy Laboratory (NREL) [19] as follows: sulfuric acid (72 wt %, 3.0 mL) was added slowly to holocellulose (300 mg) and kept at 30 °C for 1 h. The resulting solution was diluted by water (84 mL) until the concentration of sulfuric acid was 4 wt %. Acid hydrolysis was performed by autoclaving at 121 °C for 1 h. After the neutralization by CaCO_3 , the solution was subjected to a centrifugation to give the supernatant solution (ca. 87 mL), which was concentrated to 30 mL by evaporation. The solution was analyzed by HPLC. The peaks of glucose and xylose appeared whereas the peaks of galactose and arabinose were very weak. The amounts of glucan and xylan were determined from the amounts of glucose and xylose determined by HPLC. It was confirmed that the total amounts of glucan and xylan were equal to the amounts of holocellulose. The ash component in lignocellulose was obtained by burning lignocellulose (2.0 g) in an electric furnace (KBF784N1, Koyo, Nara, Japan) for 2 h at 850 °C. Thus, the chemical components of lignocelluloses were determined, as shown in Table 1.

3.2. Hydrolytic Enzyme and Preparation of the Inoculum Culture of *S. cerevisiae*

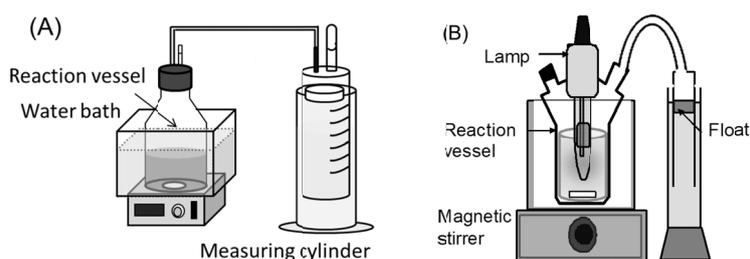
A cellulase from *Acremonium cellulolyticus* (Acremozyme KM, Kyowa Kasei, Osaka, Japan) was used [3,20]. The cellulase activity of Acremozyme was determined to be 1320 units/mg by the method of breaking down filter paper [20]. A xylanases from *Trichoderma longibrachiatum* (reesei) (Sumizyme X, Shin Nihon Chemicals, Anjo, Japan, 5000 u/g) was selected from commercially available hemicellulase. *S. cerevisiae* NBRC 2044 was cultured at 30 °C for 24 h in a basal medium (initial pH 5.5) consisting of glucose (20 $\text{g}\cdot\text{L}^{-1}$), bactotryptone (1.0 $\text{g}\cdot\text{L}^{-1}$, Difco, Leeuwarden, The Netherlands), yeast extract (1 $\text{g}\cdot\text{L}^{-1}$), NaHPO_4 (1 $\text{g}\cdot\text{L}^{-1}$), and MgSO_4 (3 $\text{g}\cdot\text{L}^{-1}$) [20]. After incubating for 24 h, the cell suspension solution of *S. cerevisiae* was obtained to use in SSF process.

3.3. Procedures of SSF

The SSF was performed using the apparatus shown in Figure 5A. A cellulosic material and buffer solution (37.5 mL) were introduced in the reaction vessel and then autoclaved at 121 °C for 20 min. After cooling to room temperature under UV-irradiation, the hydrolytic enzyme dissolved in an acetate buffer solution (22.5 mL) and the cell suspension of *S. cerevisiae* were added to the suspension of the cellulosic material. The holocelluloses from the bamboo, rice straw, and silvergrass were set to 6.04 g, 3.90 g, and 3.75 g, respectively, which corresponded to the amounts of holocellulose in 10 g of the non-treated lignocellulose. After the air was purged with N_2 , the SSF was initiated by stirring the

solution vigorously with a magnetic stirrer at an optimal temperature of 34 °C. The evolved CO₂ was collected over water by a measuring cylinder, and the reaction was monitored by the volume of CO₂. The amounts of hexose and pentose were analyzed by a Shimadzu LC-20AD (Kyoto, Japan) high-performance liquid chromatography system using anion exchange column (Shodex Asahipak NH2P-50 4E, Tokyo, Japan). Ethanol concentrations were determined by a Shimadzu GC-2014 gas chromatograph using a glass column of 5% Thermon 1000 on Sunpak-A (Shimadzu, Kyoto, Japan) with 2-propanol as an internal standard.

Figure 5. The apparatus for (A) SSF and (B) photo-Reform.



3.4. Procedures of Photo-Reform

The photo-Reform was performed as follows. The Pt-TiO₂ (100 mg) and the supernatant solution of xylose (0.25–1.25 mmol) were introduced into the reaction vessel, which was attached to a measuring cylinder with a gas-impermeable tube to collect the evolved gas (Figure 5B). After that, the volume of the solution was adjusted to 150 mL by adding water, and a high-pressure mercury lamp (100 W, UVL-100HA, Riko, Funabashi, Japan) was inserted into the reaction vessel. The reaction vessel was set in a water bath to keep it at a constant temperature (usually 20 °C). After the O₂ was purged from the reaction vessel by N₂ gas, irradiation was performed while being vigorously stirred with a magnetic stirrer. The evolved gas was collected by the measuring cylinder to measure the volume of the evolved gas. The evolved gas was analyzed on a Shimadzu GC-8A equipped with a TCD detector at a temperature raised from 40 °C to 180 °C using a stainless column (Φ3 mm × 6 m) packed with a SHINCARBON ST (Shimadzu). H₂ and CO₂ were detected in addition to N₂ which was used as the purging gas.

3.5. Preparation of Photocatalyst

An anatase-type of TiO₂ (ST-01) was purchased from Ishihara Sangyo Kasei Ltd, Osaka, Japan. According to previous research [21], the Pt-TiO₂ was prepared by irradiation of a deaerated aqueous solution (400 mL) containing TiO₂ (4.0 g), K₂PtCl₆ (20–200 mg), and 2-propanol (2.4 g, 3.0 mL) by a high-pressure mercury lamp for 24 h under stirring. Water was entirely removed from the photolysate by an evaporator. The resulting precipitate was washed with water on a filter and then dried under reduced pressure to produce the Pt-TiO₂. In our previous study, the optimized Pt-content of TiO₂ was determined to be 2.0 wt % [16].

4. Conclusions

The SSF process poses an advantage in ethanol-production from the standpoint of simplicity of the manufacturing process. The present study showed that xylose derived from SSF of lignocellulose

could be efficiently transformed into hydrogen. The formed bio-fuel, ethanol and hydrogen, has almost the same combustion energy as the saccharide occurring in lignocelluloses. If the UV light in sunlight is used as the light source for catalytic reaction, this will provide a useful method to produce H₂ from biomass.

Acknowledgments

This study was partially supported by a Grant-in-Aid for Scientific Research (C) No. 24610055 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Author Contributions

Masahide Yasuda conceived experimental idea and analyzed experimental data. Also Masahide Yasuda prepared the manuscript and revised it. Ryo Kurogi carried out SSF and photo-Reform. Hikaru Tsumagari carried out the preparation of Pt-loaded TiO₂ catalyst as well as photo-Reform using catalyst repeatedly used under supervision of Tsutomu Shiragami. Tomoko Matsumoto carried out a measurement of an X-ray diffraction.

Conflicts of Interest

The authors declare no conflict of interest.

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