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The effect of TiO_2-photocatalytic pretreatment on the biological production of ethanol from lignocelluloses

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ABSTRACT

In biological production of EtOH from lignocellulose such as napiergrass (*Pennisetum purpureum* Schumach; **1a**) and silver grass (*Miscanthus sinensis* Anderss, **1b**), the effect of the photocatalytic pretreatment with TiO_2 (PC-pretreatment) to enzymatic saccharification by *Acremozyme* cellulase (SA-reaction) and fermentation by *Saccharomyces cerevisiae* (FE-reaction) was examined. The PC-pretreatment was remarkably effective for the shortening of the reaction time in the SA and FE reactions compared with the other pretreatments such as no pretreatment (NO-treatment) and pretreatment with NaOH (AL-pretreatment). However, the PC-pretreatment did not affect to the final product distribution. The PC → SA → FE process converted holocellulose (44 g) of **1a** to 7.5 g of EtOH. Also, the PC → SA → FE process of holocellulose (41 g) of **1b** produced EtOH in 5.5 g.

1. Introduction

Production of ethanol from biomass has been receiving a great amount of interest from the stand points of utilization of renewable resources [1]. Commercially available bio-ethanol has been prepared from the starch of maize, sugarcane and sugar sorghum [2]. However, these materials are in competition with food sources for human consumption. Among many kinds of bio-ethanol sources, we are interested in herbaceous lignocelluloses such as napiergrass (*Pennisetum purpureum* Schumach; **1a**) [3] and silver grass (*M. sinensis* Anderss, **1b**). Enzymatic saccharification and fermentation are mostly used for bio-ethanol production form cellulose materials [4–6]. Lignocellulosic biomass was composed of cellulose and hemicellulose components and lignin components (Lg) which strongly connected each other [7]. For the efficient biological saccharification of lignocellulosic materials, the pretreatments by dilute sulfuric acid [8,9], alkali [10], and pressured hot water [11] have been applied. Recent requirement for the pretreatments trends to environmentally conscious process as well as efficiency of saccarification [12]. Photocatalytic reaction is expected to be met the recent requirements, since it has been utilized as environmentally conscious process in variety of filed [13]. However, the photocatalytic pretreatment (PC-pretreatment) of cellulose materials has been scarcely reported except for the application to degradation of Lg and related compounds isolated from lignocellulose [14,15]. Here, we have investigated the effect of PC-pretreatment of **1** using the TiO_2 under UV-irradiation on the biological production of ethanol from **1**. The effects of PC-pretreatments were compared with the alkali-treatment (AL-treatment) and no-treatment (NO-treatment) from the stand points of yield and reaction time (Scheme 1).

2. Materials and methods

2.1. Lignocellulose material

Dwarf type of napiergrass (*P. purpureum* Schumach; dwarf variety of late-heading type, **1a**) [3] was cultivated in the Sumiyoshi Ranch, Faculty of Agriculture, University of Miyazaki. Naturally grown silver grass (*M. sinensis* Anderss, **1b**) was collected from the campus. Leaf and stem parts of these soft-celluloses were cut by a cutter and powdered by a Wonder Blender WB-1. Amorphous cellulose powder (**1c**) was purchased from Nakarai tesque Inc as a reference sample.

2.2. Analysis

The amounts of holocellulose (2; sum of cellulose and hemicellulose) and Lg components occurring in the **1** were determined as follows: Dried **1** was powdered by a cutter until 75 wt% of particles

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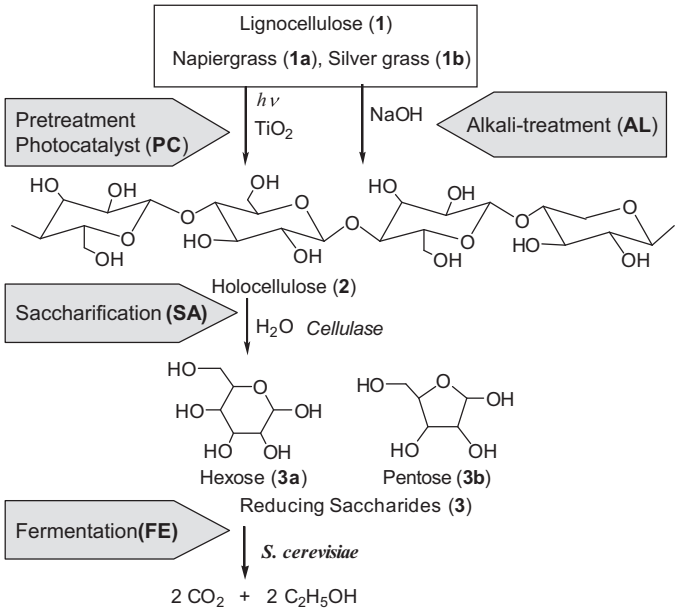


Scheme 1. Transformation from lignocellulose (**1**) to EtOH through the pretreatments followed by saccharification and fermatation.

were below 150 μm-size. The powdered **1** (30 g) was treated in a 1% aqueous solution of NaOH (400 ml) at 95 °C for 1 h. The **2** was isolated as a pale yellow precipitate from the treated solution by centrifugation. The supernatant solution was neutralized to pH 5.0 by a dilute HCl solution. The resulting dark brown precipitates (Lg) were collected by centrifugation at 10,000 rpm for 10 min.

The amount of the reducing sugars (**3**) isolated by enzymatic treatment was analyzed by the modified Somogyi–Nelson method [16], assuming the composition of **3** as $C_6H_{12}O_6$. The amounts of pentose (**3b**) were analyzed by a modified orcinol method using 5-methylresorcinol (orcinol), $FeCl_3{\cdot}5H_2O$, and conc. HCl [17]. The amounts of hexose (**3a**) were obtained by the subtraction of weight of **3b** from weight of **3**. EtOH formed by ethanol-fermentation was analyzed on a Shimadzu CTO-10A high performance liquid chromatograph equipped with a Shodex SP 0810 column and a Shimadzu RID-10A refractive index detector using water as eluent.

2.3. TiO_2-photocatalytic pretreatment (PC-pretreatment)

TiO_2 (particle size: 7 nm, surface area: 300 $m^2\,g^{-1}$, ST-01, Ishihara Sangyo Kasei Ltd.) with photocatalytic activity was used. The powdered **1** (1 g) was mixed with TiO_2 (0.1 or 0.2 g) for 15 min by a Retsch PM100 ball mill. The mixed samples were spread out in thin layer on a glass plate and covered by another glass plate. The **1** and TiO_2 mixed samples were irradiated in solid state from above the plates by a black-light blue fluorescent lamp (λ = 360 nm, 1.0 W, Panasonic FL8BL-B). The mixed samples were subjected to saccharification without separation of TiO_2 from the samples.

2.4. Alkali-treatment (AL-treatment)

The powdered **1** (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 **2** was isolated as a pale yellow precipitate from the treated solution by centrifugation.

2.5. Saccharification with cellulase

At first, cellulose was selected from four kinds of cellulases: *Acremozyme* (Kyowa kasei), *Meycellase* (Kyowa kasei), a cellulase from *Trichoderma viride* (Wako chemicals) and a cellulase from *Aspergillus niger* (Fluka). The saccharification of the powdered **1a** (200 mg) by cellulose (20 mg) was performed in an acetate buffer solution (20 ml, pH 5.0) undervigorous shaking at 45 °C for 48 h. The solution was subjected to centrifugation at 12,000 rpm to give the supernatant solutions which was subjected to analysis of **3**. As a result, *Acremozyme* was most effective among the cellulases tested. Therefore, *Acremozyme* was used for the saccharification of **1** throughout this study.

2.6. Ethanol fermentation

S. cerevisiae NBRC 2044 was cultured under nitrogen atmosphere at 30 °C for 24 h in a basal medium (pH 5.5) consisting of glucose (20 g dm^{-3}), bactotryptone (1.0 g dm^{-3}, Difco), yeast extract (1 g dm^{-3}), $NaHPO_4$ (1 g dm^{-3}), and $MaSO_4$ (3 g dm^{-3}). After the incubation for 24 h, the cell suspension solution of *S. cerevisiae* was obtained. The suspension solution (0.16 ml) of *S. cerevisiae* was added to a portion (8 ml) of solution **3** which was taken from the solution (60 ml) obtained from the saccharification of **1**. The fermentation was performed at 30 °C up to 48 h with stirring with magnetic stirrer. The evolved CO_2 was collected over water by messcylinder and the volume was measured.

3. Results

3.1. Component analysis of *1*

The dried **1a** was composed of 44.0 wt% of **2** and 19.7 wt% of Lg. 41.0 wt% of **2** and 21.7 wt% of Lg were obtained from the dried **1b**. 36.4 wt% and 37.3 wt% of water-soluble unknown components such as silica and amino acids amounts remained in the aqueous solution for **1a** and for **1b**, respectively.

3.2. Enzymatic saccharification

The AL-treatment is a popular method to separate Lg from lignocellulose and has been used as the pretreatment to promote the enzymatic saccharification (SA-reaction) [10]. The Lg-removed the **2** (4.40 g and 4.10 g) obtained from the AL-treatment of **1** (10 g) were subjected to SA-reaction by *Acremozyme* (1.0 g) in an acetate buffer solution (60 ml) at 45 °C. Table 1 shows the yields of the reducing sugars (**3**) based on **2** at the given saccharification time (T_{SA}). The yields of **3** reached the maximum yield (60.8%) after the SA-treatment for 48 h of the Lg-removed **2** from **1a** (run **1**). Direct saccharification of the powdered **1** (10 g) by *Acremozyme* (1.0 g) in a buffer (60 ml) at 45 °C for 48 h gave **3** in 61.4% yield (NO treatment, run **2**). It was confirmed that the co-existence of Lg did not affect the yields of SA-reaction, since the yields of **3** was almost same as in the cases of the AL- and NO-pretreatments. In the case of **1b**, **3** was formed in 55.5% and 39.0% yields by the SA-reaction for 48 h of the AL-pretreated (run **6**) and the NO-treated **1b** (run **7**), respectively. Therefore, the AL-treatment was effective for the case of **1b**.

Time-conversion plots of SA-reaction of the AL- and NO-pretreated **1a** and **1b** are shown in Fig. 1. The SA-reaction of the AL- and the NO-pretreated **1** proceeded slowly to require a longer T_{SA} (>48 h).

3.3. Effects of photocatalytic pretreatment on saccharification

TiO_2-photocatalytic pre-treatments (PC-pretreatment) was performed by the irradiation of the mixture of the powdered **1**

Table 1
Effects of the pretreatments for saccharification and fermentation of **1**.

Run no.	**1**[a]	Pretreatment	Saccharification (SA)[b]										Fermantation (FE)[c]			
			Yields of **3**/%[d]									T_{SA}^{80}/h[f]	Conv.[g]	Yields (%)		T_{FE}/h[j]
			T_{SA}/h = 0	1	3	6	9	12	24	**3**$_{Max}$	(**3a**:**3b**)[e]			EtOH[h]	CO_2[i]	
1	**1a**	AL[k]	1.6	16.6	22.5	30.7	37.0	39.3	60.0	60.8	(0.79:0.21)	17	63.0	98.7	110.2	46
2	**1a**	NO[l]	7.3	27.7	30.4	34.5	37.7	50.3	55.9	61.4	(0.75:0.25)	24	86.3	88.5	98.9	23
3	**1a**	PC(3)[m]	5.4	56.4	57.7	61.8	60.5	64.5	64.1	63.0	(0.67:0.33)	0.5	82.2	85.8	108.9	18
4	**1a**	PC(2)[m]	6.4	51.4	53.6	57.3	65.5	68.2	66.8	66.8		3				
5	**1a**	PC(1)[m]	6.4	36.8	48.6	54.0	55.4	60.5	62.7	62.7		6				
6	**1b**	AL[k]	1.4	24.1	29.3	34.9	37.8	43.9	58.3	55.5	(0.80:0.20)	15	81.7	82.1	103.5	53
7	**1b**	NO[l]	3.9	12.6	17.6	23.4	23.9	26.8	33.6	39.0	(0.78:0.22)	24	81.5	84.2	97.6	23
8	**1b**	PC(3)[n]	5.4	33.6	40.5	41.0	42.0	41.5	40.0	41.0	(0.78:0.22)	1	81.4	82.1	92.0	14
9	**1b**	PC(2)[n]	5.5	26.3	26.8	33.7	39.5	40.0	40.0	40.0		6				
10	**1b**	PC(1)[n]	4.4	19.5	29.8	29.8	38.0	40.5	39.5	40.5		8				

[a] Napiergrass (**1a**) contained 44.0 wt% of **2** and 19.7 wt% of Lg. Silver grass (**1b**) contained 41.0 wt% of **2** and 21.7 wt% of Lg.
[b] The pretreated **1** (10 g) was subjected to the saccharification by *Acremozyme* (1.0 g) in an acetate buffer solution (60 ml, pH 5.0) at 45 °C for a given saccharification (T_{SA}).
[c] The SA-treated solutions (8 ml) were fermented by *S. cerevisiae*.
[d] Yield of **3** = 100 × (weight of **3**)/(weight of **2**) × (162/180).
[e] Yields of **3** after the saccharification for 48 h. The values in parenthesis are the ratio of the **3a** and **3b** components.
[f] The T_{SA}^{80} is the reaction time required until the saccharification yield reached to 80% of the maximum yield.
[g] Conversion of **3a** = 100 $(W_{3a}^0 - W_{3a})/W_{3a}^0$ where W_{3a}^0 and W_{3a} denote the weights of **3a** before and after SA reaction, respectively.
[h] Yields of EtOH were based on the **3a** consumed. Yields (%) = 100 × (weight of EtOH)/$(W_{3a}^0 - W_{3a})$ × (90/46).
[i] Yields of CO_2 were based on the **3a** consumed. Yields (%) = 100 × (weight of CO_2)/$(W_{3a}^0 - W_{3a})$ × (90/44).
[j] The T_{FE} is a fermentation time required until the CO_2-evolution reached the maximum yield.
[k] Alkaline-treatment was performed by heating **1** (30 g) in a 1% aqueous solution of NaOH (400 ml) at 95 °C for 1 h to give **2** in 44.0% and 41.0% for **1a** and **1b**, respectively.
[l] NO pre-treatment.
[m] The photocatalytic pretreatment (PC pretreatment) was performed for **1** (10 g) with TiO_2 (1.0 g) under UV-irradiation. The values in parenthesis are the irradiation time (T_P).
[n] The PC-treatment was performed for **1** (10 g) with TiO_2 (2.0 g) under UV-irradiation.

(10 g) and TiO_2 (2.0 g or 1.0 g) at 360 nm for 1, 2, and 3 h of irradiation time (T_{PC}). Only PC-pretreatment formed **3** in poor yields (<6.4%). In same conditions as the case of NO-treated **1**, the SA-reaction of the PC-pretreated **1** (10 g) was performed with *Acremozyme* (1.0 g) in a buffer solution (60 ml) (Table 1, runs 3–5 and 8–10). The SA-reaction of the PC-pretreated **1a** and **1b** for 3 h give **3** in 63.0% and 41.0% of yields at the maximum, respectively (runs **3** and **8**). However, the SA-reaction of the mixture of **1** and TiO_2 without irradiation were inefficient. These values were very similar to the cases of the NO-pretreatment.

Fig. 2 shows time conversion plots in the SA-reaction of the PC-pretreated **1**. The **2** increased near the maximum yield immediately after the reaction started. The effects of PC-pretreatment on the SA-reaction were evaluated by the saccharification time (T_{SA}^{80}) required until the SA-reaction reached 80% of the maximum yield. The T_{SA}^{80} was getting shorter with an increasing T_{PC}. In the case of T_{PC} for 3 h, the T_{SA}^{80}s were determined to be 0.5 h and 1.0 h for **1a** and **1b**, respectively. The T_{SA}^{80} value in the case of the PC-pretreatment was extremely shorter than the 15–17 h in the case of the AL-pretreatment and 24 h in the case of NO-treatment. Thus, it was found that the PC-pretreatment remarkably accelerated the saccharification.

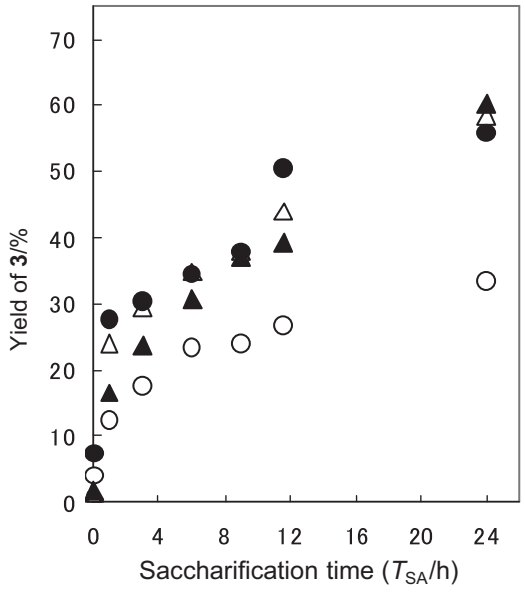


Fig. 1. Time-conversion plots of saccharification of the NO-treated (●) and the AL-pretreated **1a** (▲) as well as the NO-treated (○) and the AL-pretreated **1b** (△).

3.4. Effects of PC pretreatment on fermantation

Saccharides solutions (60 ml) were obtained from each PC → SA, AL → SA, and NO → SA processes of **1** (10 g). A portion (8 ml) of the saccharide solutions (33.7–92.3 g l^{-1} of **3**) underwent to the EtOH-fermentation at 30 °C using a *S. cerevisiae* (yeast) suspension (0.16 ml) (FE reaction). The reaction procedure was monitored by CO_2-evolution. Fig. 3 shows the time conversions of CO_2-evolutions in the fermentation of **3** produced by the PC → SA, AL → SA, and NO → SA processes of **1** (10 g). The evolution amounts of CO_2 showed stoichiometrically good agreement with the yield of EtOH, as shown in Table 1. The **3b** was not consumed by the FE-treatment at all, showing that no formation of EtOH from **3b** took place. Therefore, the yield of EtOH was determined based on **3a**.

At the maximum evolution of CO_2, the yield of EtOH was 85.8% based on the consumed **3a** which was obtained from the PC → SA process of **1a**. This EtOH yield was similar to the yields (98.7% and 88.5%) in the FE-reactions of **3a** obtained from the AL → SA and the NO → SA processes. It was confirmed that the coexistence of TiO_2 did not disturb the FE-reaction at all. In the case of **1b**, yields of EtOH were determined to be 99.0, 84.2, and 82.1% in the FE-reaction of **3a** obtained from the AL → SA, NO → SA, and PC → SA processes, respectively. Thus, the pretreatment affected scarcely the EtOH yields.

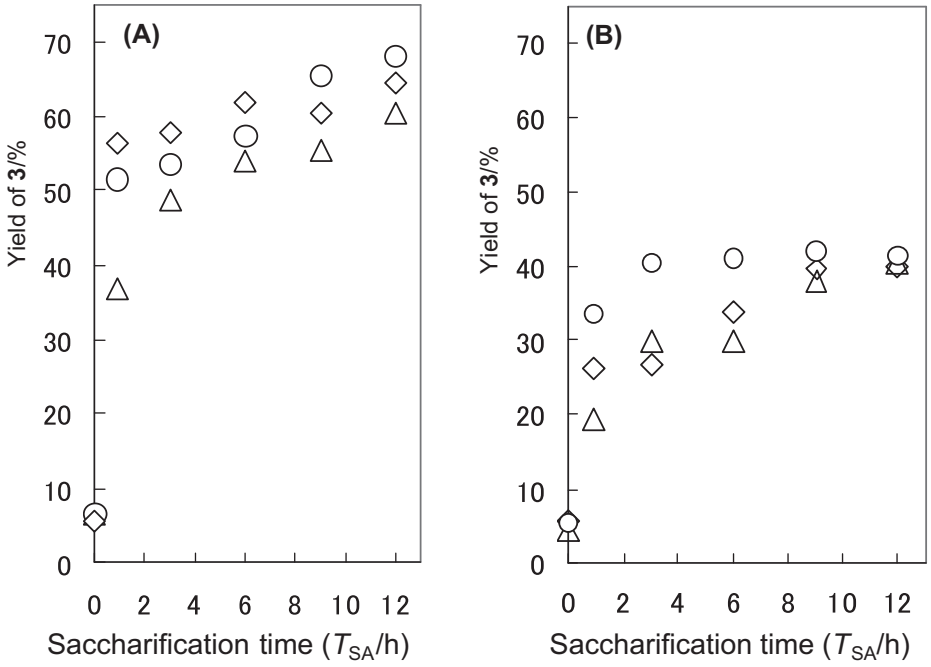


Fig. 2. Saccharification of the PC-pretreated **1a** (A) and **1b** (B) under irradiation for irradiation time (T_{PC}): T_{PC} = 1 h (△), 2 h (◊), and 3 h (○).

The effects of the pretreatments on the FE-reaction were evaluated by the fermentation time (T_{FE}) to reach maximum yields of the CO_2-evolution. The T_{FE} of **3a** obtained by the PC → SA process were 18 h and 13 h for **1a** and **1b**, respectively. These T_{FE} s were much shorter compared with those in the cases of AL → SA (46 h and 53 h) and NO → SA (23 h) processes of **1a** and **1b**, respectively. Especially, it was suggested that the AL treatment slowed down the FE reaction, because the AL treatment removed the protein and mineral which was expected to serve as the nutrients for the fermentation of *S. cerevisiae*. Thus, it was found that the PC-pretreatment was effective in shortening the T_{FE} whereas the AL-pretreatment retarded the FE-reaction.

4. Discussion

4.1. Total mass balances

Table 2 summarizes the weights of products produced from the pretreatments (PC, AL, and NO) → SA → FE of 100 g of lignocellulose (**1**). In the case of **1a**, the pretreatments (PC, AL, or NO) → SA process gave 20.8–23.3 g of **3a** which was subjected to FE-reaction to produce 7.4–8.8 g of EtOH along with the formation of 7.9–9.4 g of CO_2. In the case of **1b**, **3a** in 21.8 g was produced from the AL → SA process of **1b** (100 g) which was larger than the 15.7 and 16.1 g of the PC → SA and NO → SA processes. 8.8 g of EtOH was formed

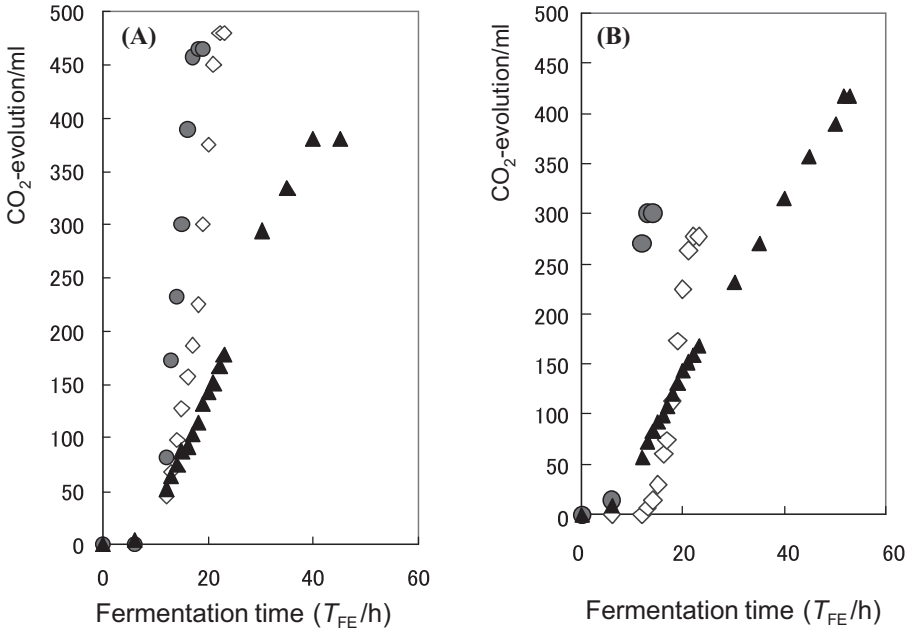
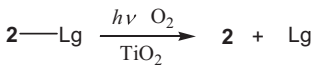


Fig. 3. The CO_2-evolutions in the fermentation of **3** produced by the PC → SA (●), AL → SA (▲), and NO → SA (◊) processes of 10 g of **1a** (A) and **1b** (B).

Table 2
Distribution of products formed by pretreatment (AC, NO, or PC) → SA → FE reactions of lignocellulose (**1**).

Pretreatments[a]		Products/g[b]					T/h[c]
		2	**3a**	**3b**	EtOH	CO_2	
	AL	19.2	8.6	6.4	7.4	7.9	63
1a[d]	NO	17.0	3.1	7.1	8.8	9.4	47
	PC	16.3	3.7	10.1	7.5	9.1	18.5
	AL	18.2	4.0	5.1	8.8	8.2	68
1b[e]	NO	25.0	2.9	3.9	5.5	6.1	47
	PC	24.2	3.0	4.1	5.5	5.9	15

[a] AL: pretreatment with aq. NaOH solution; NO: no pretreatment; PC: pretreatment by TiO_2 for 3 h-irradiation.
[b] Product amounts after the pretreatment → SA → FE reactions of 100 g of 1.
[c] Sum of T_{SA}^{80} and T_{FE}.
[d] Napiergrass (**1a**) contained 44.0 wt% of **2** and 19.7 wt% of Lg.
[e] Silver grass (**1b**) contained 41.0 wt% of **2** and 21.7 wt% of Lg.



Scheme 2. TiO_2-photoaccelerated fission of the linkage between **2** and Lg.

by the AL → SA → FE process whereas 5.5 g of EtOH was formed through the PC and NO → SA → FE processes. The AL pretreatment was effective in the case of **1b**. Total reaction times (T), sum of T_{SA}^{80} and T_{FE}, were 18.5 and 15 h for the case of PC → SA → FE process, while T were 63–68 h and 47 h for the cases of AL → SA → FE and NO → SA → FE processes, respectively.

4.2. Effect of PC-pretreatment

The PC-pretreatment had no effects for amorphous cellulose powder (**1c**) which contained no Lg component, since its T_{SA}^{80} was the same as that of the NO-pretreatments of **1c**. It has been reported that a hydroxyl radical generated by TiO_2 under irradiation retarded the Lg and related compounds [14]. In the present case, therefore, it is suggested that the TiO_2 oxidize the phenolic moiety of the Lg under irradiation to accelerate the fission of the linkage between **2** and Lg (Scheme 2). Moreover, the ratio of **3b** to **3a** in **3** obtained from the PC → SA process was larger than those from the AL → SA and NO → SA treatments (run **3** in Table 1). Hemicellulose part which consisted with the polymer of **3b** in plants was located near Lg component rather than cellulose part. It is suggested that bond fission between hemicellulose and Lg parts was accelerated by PC-pretreatment.

As a conclusion, the PC pretreatment did not affect to the final product distribution, showing that TiO_2 did not disturb the biological reactions by the cellulase and yeast. Moreover, the PC-pretreatment extremely shortened the reaction time (T_{SA} and T_{FE}) of the biological reactions. Thus, the PC-pretreatment are an environmentally conscious process without acid and alkali. This is a first finding on the PC-pretreatment which includes an important knowledge for EtOH-production process of soft-cellulose.

References

[1] O.P. Ward, A. Singh, Bioethanol technology: development and perspectives, Advances Applied Microbiology 51 (2002) 53–80.
[2] M. Balat, H. Balat, Recent trends in global production and utilization of bio-ethanol fuel, Applied Energy 86 (2009) 2273–2282.
[3] Y. Ishii, M. Mukhtar, S. Idota, K. Fukuyama, Rotational grazing system for beef cows on dwarf napiergrass pasture oversown with Italian ryegrass for 2 years after establishment, Grassland Science 51 (2005) 209–220.
[4] M. Balat, H. Balat, C. Öz, Progress in bioethanol processing, Progress Energy Combustion Science 24 (2008) 551–573.
[5] M.G. Tabka, I. Herpoël-Gimbert, F. Mond, M. Asther, J.C. Sigoillot, Enzymatic saccharification of wheat straw for bioethanol production by a combined cellulose xylanase and feruloyl esterase treatment, Enzyme Microbial Technology 39 (2006) 897–902.
[6] B.S. Dien, H-J.G. Jung, K.P. Vogel, M.D. Casler, J.F.S. Lamb, L. Item, R.B. Mitchell, G. Sarath, Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass, Biomass & Bioenergy 30 (2006) 880–891.
[7] J. Zaldivar, J. Nielsen, L. Olsson, Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration, Applied Microbiology Biotechnology 56 (2001) 17–34.
[8] Y. Sun, J.J. Cheng, Dilute acid pretreatment of rye straw and bermudagrass for ethanol production, Bioresource Technology 96 (2005) 1599–1606.
[9] C. Martin, B. Alriksson, A. Sjöde, N. Nilvebrant, L. Jönesson, Dilute sulfuric acid pretreatment of agricultural and agro-industrial residues for ethanol production, Applied Microbiology Biotechnology 137–140 (2007) 339–352.
[10] H.B. Klinke, B.K. Ahring, A.S. Schmidt, A.B. Thomsen, Characterization of degradation products from alkaline wet oxidation of wheat straw, Bioresource Technology 82 (2002) 15–26.
[11] B.S. Dien, X.-L. Li, L.B. Iten, D.B. Jordan, N.N. Nichols, P.J. O'bryan, M.A. Cotta, Enzymatic saccharification of hot-water pretreated corn fiber for production of monosaccharides, Enzyme Microbial Technology 39 (2006) 1137–1144.
[12] H. Li, N.-J. Kim, M. Jiang, J.W. Kang, H.N. Chang, Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid-acetone for bioethanol production, Bioresource Technology 100 (2009) 3247–3251.
[13] A. Fujishima, T.N. Rao, D.A. Tryk, Titanium dioxide photocatalysis, Journal Photochemistry Photobiology C: Reviews 1 (2000) 1–21.
[14] A.E.H. Machado, A.M. Furuyama, S.Z. Falone, R. Ruggiero, DdS. Perez, A. Castellan, Photocatalytic degradation of lignin and lignin models, using titanium dioxide: the role of the hydroxyl radical, Chemosphere 40 (2000) 115–124.
[15] B.N Kuznetsov, V.G. Danilov, S.A. Kuznetsova, O.V. Yatsenkova, N.B. Alelsandrova, Optimization of fir wood delignification by acetic acid in the presence of hydrogen peroxide and a TiO_2 catalyst, Theoretical Foundations of Chemical Engineering 43 (2009) 499–503.
[16] Y.-K. Kim, Y. Sakano, Analyses of reducing sugars on a thin-layer chromatographic plate with modified Somogyi and Nelson reagents, and with copper bicinchoninate, Bioscience Biotechnology Biochemistry 60 (1996) 594–597.
[17] W.R. Fernell, H.K. King, The simultaneous determination of pentose and hexose in mixtures of sugars, Analyst 78 (1953) 80–83.