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An integrated process for continuous cellulosic bioethanol production

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Abstract−A high-efficiency, integrated bioethanol production process was developed in this study, using *Miscanthus* as lignocellulosic biomass. The continuous process involved a twin-screw extruder, a pretreated biomass washing/dewatering process, and a saccharification/fermentation process. In addition, the integration process was designed for the reuse of pretreatment solution and the production of highly concentrated bioethanol. Pretreatment was performed with 0.72 M NaOH solution at 95 °C using an 80 rpm twin-screw speed and a flow rate of 90 mL/min (18 g/min of raw biomass feeding). Following washing and dewatering steps, the pretreated biomass was subjected to simultaneous saccharification and bioethanol fermentation processes. The maximum ethanol concentration, yield from biomass, and total volume obtained were 59.3 g/L, 89.9%, and 60 L, respectively, using a pretreated biomass loading of 23.1% (w/v) and an enzyme dosage of 30 FPU/g cellulose. The results presented here constitute an important contribution toward the production of bioethanol from *Miscanthus*.

Keywords: *Miscanthus*, Twin-screw Extruder, Reuse of Pretreatment Solution, Integration Process, Simultaneous Saccharification and Fermentation (SSF)

INTRODUCTION

Given the global rise in energy demand and concerns regarding increased greenhouse gas emissions, lignocellulosic biomass is being increasingly recognized as having great potential for biofuel and biomaterial production in biorefineries [1,2].

Miscanthus is a key lignocellulosic biomass crop with relatively low maintenance requirements and high yield/energy content, and it thus plays an important role in the sustainable production of renewable fuels and chemicals via biochemical conversion [3,4]. *Miscanthus* can grow to a height of 4 m [5], and the height of the plant is dependent on the species and growth conditions. The biomass yield of *Miscanthus* depends on the season in which it is harvested [6]. In northern Europe, *M. sinensis* hybrids have yielded up to 25 t/ha, whereas in middle and southern Europe, *M. giganteus* yields up to 38 t/ha and *M. sinensis* hybrids yield up to 41 t/ha [7].

Lignocellulose normally consists of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose can be converted to sugars through biological and chemical reactions, and these sugars can be fermented to ethanol or other valuable chemicals [8]. However, lignin inhibits the function of cellulase, thereby blocking cellulose hydrolysis [9]. Thus, pretreatment is one of the key steps in the bioconversion of lignocellulosic materials. The goal of any pretreatment process for bioethanol production is to remove structural and compositional obstacles to hydrolysis, to improve the rates of enzyme digestion, and to increase the yields of fermentable sugars from substrates [10]. Pretreatment is required to modify the chemical structure of the lignocellulosic biomass components and make them more accessible to enzymes that convert carbohydrate polymers into fermentable sugars [3,11]. A successful pretreatment must satisfy the following conditions: (i) it improves sugar formation or the ability to subsequently form sugars by hydrolysis, (ii) it prevents the degradation of carbohydrates, (iii) it prevents the formation of fermentation by-products, and (iv) it is cost-effective [12]. Several pretreatment strategies have been investigated, including those involving hydrothermal [13], dilute acid [14], ammonia fiber expansion [15], aqueous ammonia percolation [16], steam explosion [17], and complex treatments [18]. In addition, pretreatment methods using NaOH, which effectively removes lignin, have been studied recently [19]. Because of this capacity for lignin removal, NaOH pretreatment was selected for further study here in the development of a continuous process.

Twin-screw extrusion technology is commonly used in the polymer and food industries and has many advantages as a highly cost-effective production process. Twin-screw extruders can be applied to continuous processes and have practical uses for large-scale production, as they can be easily temperature-controlled and provide high-efficient pulverization (through high shear forces), high throughput capacity, and adaptability to many different processes through modifications [20,21]. Therefore, pretreatment using twin-screw extrusion is being studied for various pretreatment methods and biomass types, such as for *Miscanthus* with NaOH pretreatment [22], wheat bran and soybean hull with thermo-mechanical treatment [23], and woody biomass with hot-compressed water pretreatment [21].

Pretreated biomass requires washing and dewatering steps prior to enzymatic hydrolysis or processing by simultaneous saccharification and fermentation (SSF). To enhance cost-effectiveness, a washing and dewatering process was developed to enable reuse of the pretreatment solution. This equipment was designed to connect

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the pretreatment and SSF processing equipment, so that both processes were combined into a continuous, integrated process.

The purpose of this study was to enhance the economic efficiency of bioethanol production by reusing pretreatment solutions involved in the integration process. The immediate goal was to reduce the cost of the pretreatment process and minimize the generation of wastewater by recycling the solvent used for pretreatment. Although pretreatment processes using NaOH are efficient, the solute is expensive. In this regard, the reuse of NaOH can increase the economic efficiency by reducing the cost of pretreatment and the amount of wastewater produced. The second goal was to enhance the economic efficiency by reducing the energy costs for distillation. When the ethanol concentration after fermentation is less than 7% (v/v), extensive energy must be consumed in subsequent distillation processes. However, it is difficult to produce ethanol with a concentration greater than 7% (v/v) from lignocellulosic biomass through conventional fermentation processes [24]. Thus, in the present study, high-concentration bioethanol was produced using a continuous integration process involving SSF.

INTEGRATION PROCESS (FIG. 1 & 2)

1. Pretreatment Reactor

A continuous, twin-screw ChangHae Ethanol Multi ExTruder (CHEMET; Changhae Ethanol Co., Jeonju, Korea) was used for pretreating lignocellulosic biomass. The CHEMET has a single-head configuration and a screw diameter of 28 mm (length : diameter ratio=36 : 1). The range of screw types involved included normal feed, reverse feed, normal short-helix feed, reverse short-helix feed, and mixing-element screws. The screw arrangement could be varied to suit the specific characteristics of the biomass. The CHEMET was manufactured for a maximum daily throughput of 30 kg, a temperature of 200 °C, and a twin-screw speed of 150 rpm. The temperature was controlled by high-pressure steam.

Fig. 2. Picture of bioethanol integration process.

2. Chang Hae Ethanol Scant reAgent Feeder (CHESAF) and Drum-filter Reactor

Washing and dewatering pretreated biomass is a necessary step

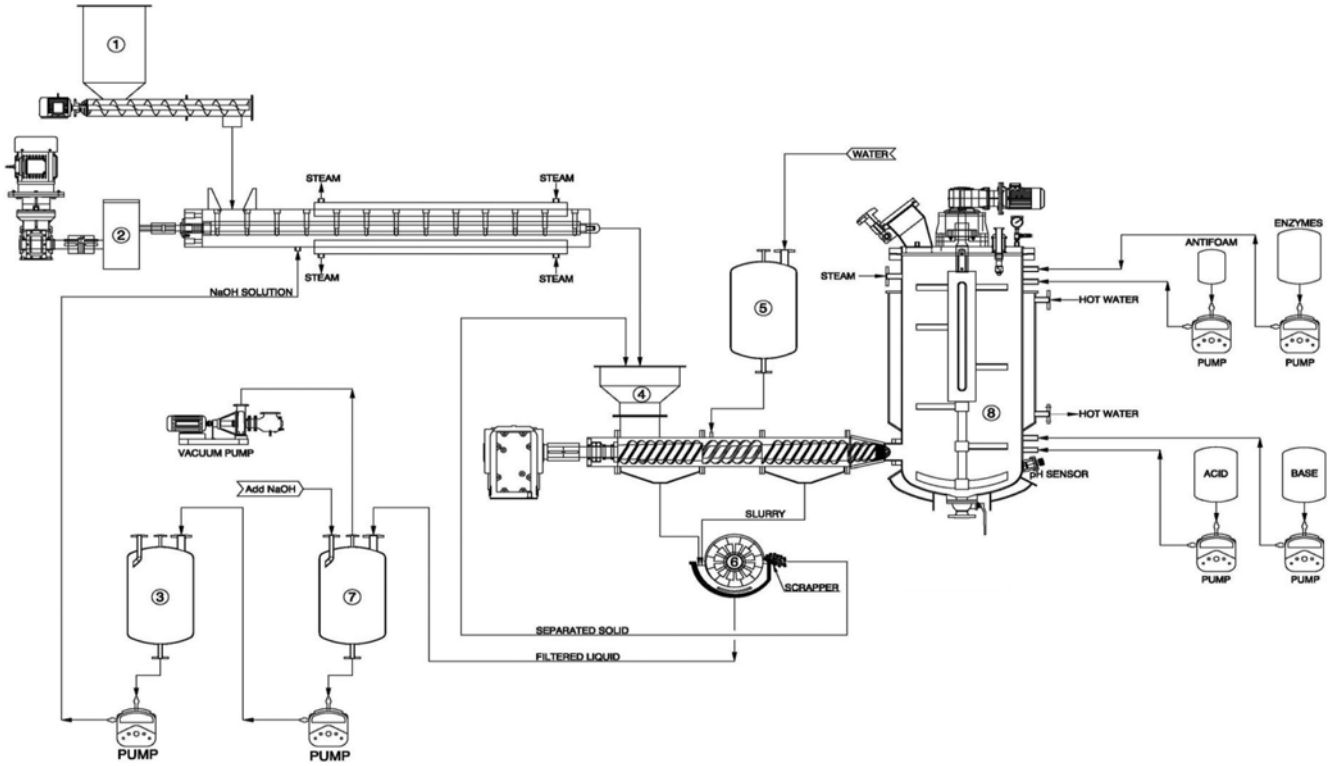


Fig. 1. Diagram of instrumentation used for efficient bioethanol production in a continuous integrated process.

1. Biomass feeder
2. CHEMET (ChangHae Ethanol Multi ExTruder)
3. 1st NaOH solution tank
4. CHESAF (ChangHae Ethanol Scant reAgent Feeder)
5. Washing water tank
6. Drum filter
7. 2nd NaOH solution tank
8. SSF (Simultaneous saccharification & fermentation) tank

that should be performed before the biomass is fed into an SSF tank. Washing and dewatering is required to clean the biomass and stably adjust the pH to an optimum for subsequent enzymatic treatment and, thus, for high-concentration bioethanol production. The CHESAF is composed of parts that control dewatering, washing, and reagent feeding with the prevention of back flow. Dewatering was achieved with a screw press that decreases the biomass volume as a screw shaft is advanced. The feeder was made from a conical-type screw that prevents back flow from the saccharification and fermentation tank. The CHESAF is very simple and efficient, as the dewatering, washing, dewatering, and feeding steps are performed within a single unit. The diameter of the screw and rotating speed are 100 mm and 1-5 rpm, respectively, in the CHESAF.

The drum filter was mounted in the bottom of the CHESAF to separate liquid and solid materials from the slurry of dewatered biomass. The separated liquid was collected for subsequent recovery of the NaOH-containing pretreatment solution, and the solid material was re-inputted to minimize biomass loss. The drum filter is contained in a drum with a 300-mm outside diameter, which can be rotated at 1-11 rpm. The filter was made from aramid fibers, which offer stability against alkali and organic compounds, thermal resistance, and a permeability of 20 ml/cm^2. The drum filter required a minimum of 10 L slurry for proper operation and was able to digest 20 L of slurry in 1 h.

3. SSF Reactor

The SSF reactor was prepared for simultaneous saccharification fermentation. The reactor was constructed from SS304 stainless steel, with dimensions of 400 mm (inside diameter)×800 mm (length) and a 100-L internal volume. Reaction temperatures in the SSF reactor were controlled with a hot water tank. The reactor was also designed to perform sterilization using high-pressure steam. The SSF reactor was designed with an entrance point for pretreated biomass feeding, a pH sensor, a level gauge, and a reagent nozzle in the underbody of the tank, as well as a hand hole, a washing line, an inoculation spot, and a vent in top of the reactor. The pH sensor located in the underside of tank enabled efficient pH measurements with pretreated biomass, indicating whether the pH should be adjusted. Agitation was performed with a 2-blade pitched paddle to facilitate vigorous mixing of highly viscous materials.

MATERIALS AND METHODS

1. Materials

Miscanthus was obtained from the Bioenergy Crop Research Center in Muan, Korea and was used as feedstock. *Miscanthus* was milled and sieved to a particle size of less than 2 mm and then stored in a plastic container at room temperature. Cellulase (Cellic® CTec2, Novozymes Korea Ltd.) and hemicellulase (Cellic® HTec2, Novozymes Korea Ltd.) were used in the SSF process. All reagents (except for NaOH) used in this study were of analytical grade. NaOH (Duksan Chemical Co. Ltd., Korea) was of industrial grade (97% purity).

2. Pretreatment for Reuse of Pretreatment Solution

The study was performed under the following conditions to enable the reuse of pretreatment solution and to minimize the amount of wastewater generated. Pretreatment conditions used were established in our previous study [25]. Briefly, 0.72 M NaOH was used as the pretreatment solution. The biomass-feeding rate was set at 18 g/min, the amount of pretreatment solution at 90 ml/min, and the ratio of solids to liquids at 1 : 5. Pretreatment was performed at 95 °C, and the rotation speed was set to 80 rpm. The biomass residence time was 4 minutes under these conditions.

Thirty liters of 0.72 M NaOH pretreatment solution was prepared for reuse experiments. The dewatered slurry was separated into liquid and solid components by filtration in drums, and the recovered liquid was transferred to a second NaOH solution tank by a vacuum pump. When the volume of the collected liquid reached 15 L, the NaOH concentration was adjusted to 0.72 M NaOH by adding the appropriate quantity of NaOH, and the resulting solution was transferred back to the first NaOH solution tank for reuse. Because the drum filter requires a minimum of 10 L of solution for stable operation, we planned on collecting >15 L of filtered liquid; thus, the pretreatment solution was manufactured at a 30-L scale at the initial stage. The same pretreatment process was used in cases where the pretreatment solution was reused.

3. SSF Process for the Production of Highly Concentrated Bioethanol

Fourteen liters of water was added to the SSF tank to enable the smooth control of pH during processing, and sterilization was performed at 100 °C for 30 min to prevent contamination. The pretreated biomass was inputted at a rate of 1.70 kg/h in the SSF reactor. In addition, 3.0 M sulfuric acid was used to adjust the pH to 5.0, which was optimal for enzymatic activation. Enzymes were added at concentrations of 30 FPU/g cellulose (Cellic® CTec2) and 15% Cellic® HTec2 (this value was based on the amount of Cellic® CTec2 loaded). Subsequently, 3 L yeast (*Saccharomyces cerevisiae* CHY 1011) was added to the SSF tank, which was prepared as described below. Finally, the working reaction volume was adjusted approximately 60 L. SSF reactions were performed for 72 h under operating conditions of 32 °C (controlled with a hot water tank) and 90 rpm.

The *S. cerevisiae* inocula were prepared by growing strain CHY 1011 on solid YPD-agar medium containing 10 g/L yeast extract, 20 g/L protease peptone, 10 g/L dextrose, and 20 g/L agar. The solid culture was incubated at 32 °C for 48 h. For subculturing, a single colony was transferred to YPD medium containing 10 g/L yeast extract, 20 g/L protease peptone, and 100 g/L dextrose and grown at 32 °C with agitation (120 rpm) for 12 h. SSF tanks were inoculated with 3 L of YPD medium containing *S. cerevisiae* prepared as just described.

4. Analytical Methods

The total solid, acid-soluble lignin, and acid-insoluble lignin contents of *Miscanthus* were determined by using standard biomass analytical procedures (National Renewable Energy Laboratory, USA) [26]. The carbohydrate content of *Miscanthus* was estimated by measuring the amount of sugars derived from hemicellulose and cellulose. The composition of hydrolysates produced by enzymatic hydrolysis and fermentation was determined by measuring glucose, xylose, and ethanol concentrations by high-performance liquid chromatography (HPLC).

The HPLC system (Waters, USA) was equipped with a Bio-Rad Aminex HPX-87P column, a guard column, an automated sampler, a gradient pump, and a refractive index detector. The flow rate

Table 1. Major components of *Miscanthus*

Component	[%]
Cellulose	38.6
Hemicellulose	17.9
Acid-insoluble lignin	23.2
Acid-soluble lignin	2.2
Ash	2.6
Other components	15.5

of the mobile phase (deionized water) was 0.6 mL/min, and the HPLC column temperature was 85 °C. Prior to HPLC injection, all samples (derived from solids or hydrolysates) were neutralized with calcium carbonate, centrifuged at 5,000 ×*g* for 10 min, and filtered through 0.2-μm syringe filters.

RESULTS AND DISCUSSION

1. Characteristics of *Miscanthus*

The chemical composition of *Miscanthus* varies according to growth location, season, and harvesting method, and composition determinations also vary according to the analysis procedure. The composition of *Miscanthus* used in this study is shown in Table 1. Based on HPLC carbohydrate analysis, the sugar and lignin fractions were 56.5% and 25.4% (w/w), respectively. The major component of both *Miscanthus* fibers and plant cell walls was glucan (38.6%). Xylan, as the major hemicellulose constituent, constituted up to 16.5% of *Miscanthus*. Arabinan accounted for only a small portion (1.4%) of the biomass, whereas galactan and mannan were not detected. Glucan and xylan can be converted to ethanol through the use of organisms that can ferment hexose and pentose.

2. Pretreatment and Reuse of Pretreatment Solution

Pretreatment was conducted by using a twin-screw extruder to produce bioethanol from *Miscanthus*. Table 2 shows results related to the reuse of pretreatment solution, as well as the composition of the pretreated biomass used in this process. After the completion of each pretreatment step, the concentration of NaOH in the pretreatment solution was relatively constant at 0.27-0.31 M. Thus, the remaining 37.5-43.1% of the NaOH added initially could be reused for a potential cost reduction with the pretreatment reagent of at least 37.5%. In addition, wastewater production was reduced in parallel with the reuse of pretreatment solution, enabling significant cost reductions in terms of wastewater disposal as well. Therefore, the pretreated biomass composition was analyzed after reusing the pretreatment solution.

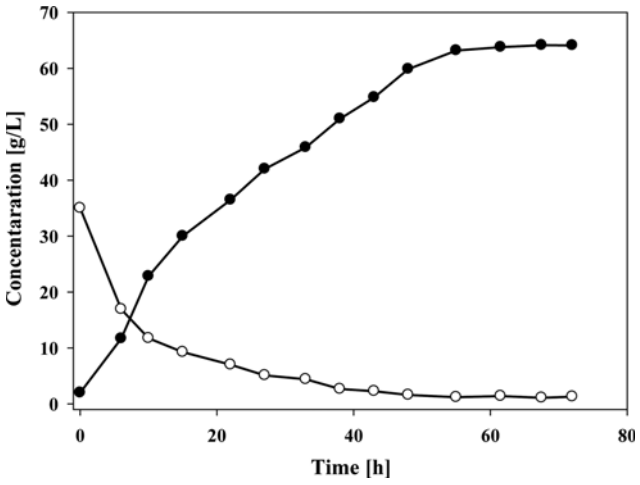


Fig. 3. Ethanol and glucose concentrations produced from pretreated *Miscanthus* at 23.1% (w/v) biomass loading with Cellic® CTec II (30 FPU/g cellulose) and Cellic® HTec II (based on 15% weight of Cellic® CTec II) enzymes, using the SSF process (○- Glucose and ●- Ethanol).

As shown in Table 2, a glucan composition of 55.2% was observed after the initial pretreatment process. After the first reuse of the pretreatment solution, the glucan composition in the processed biomass was 51.7%, indicating that the pretreatment efficiency may be reduced slightly when reusing the pretreatment solution. However, after the second reuse, the glucan component was relatively constant, with an average glucan yield of 51.6% during the second through the seventh reuse steps. The overall process was very efficient when considering the costs of pretreatment reagent and wastewater treatment.

3. SSF Process

The pretreated biomass from the CHESAF had an average mois-

Table 2. Results related to the reuse of pretreatment solution and biomass compositions after pretreatment

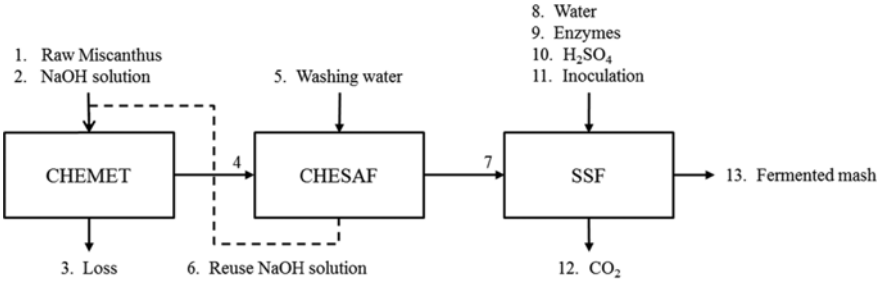
		Reuse of pretreatment solution			Composition of biomass after pretreatment			
		NaOH [M]	Added NaOH [g]	Volume [L]	Glucan [%]	Xylan [%]	Lignin [%]	Solid recovery [%]
Initial		0.72	890.7	30	55.2	13.5	15.2	56.3
Step of reuse	1	0.27	278.4	15	51.7	14.8	15.4	55.6
	2	0.31	253.6	15	50.7	15.0	17.3	57.2
	3	0.29	266.0	15	50.5	15.1	17.2	56.4
	4	0.28	272.2	15	53.0	17.2	15.6	55.9
	5	0.28	272.2	15	52.6	16.8	15.9	56.1
	6	0.27	278.4	15	51.6	17.0	15.7	55.4
	7	0.28	272.2	15	51.4	17.1	15.4	56.2
Average (after reuse)					51.6	16.1	16.1	56.1

ture content of approximately 63% (w/w). This biomass was injected into the side of the bottom part of the SSF tank through the CHESAF apparatus. Injected biomass was transformed into glucose after enzymatic hydrolysis, which in turn was converted into ethanol by added yeast. Fig. 3 shows the concentrations of glucose and ethanol detected during the SSF process. Early glucose concentration peaked at 35 g/L and decreased rapidly thereafter, which likely reflects a faster rate of glucose metabolism by yeast to generate ethanol than that of glucose production performed by enzymatic digestibility. The highest concentration of ethanol produced was 64.0 g/L. Nevertheless, this concentration of ethanol was not generated solely from the pretreated biomass. Some ethanol was also generated from glucose of enzyme stock solution and inoculation. Table 3 shows experimental parameters involved in the SSF process, as well as the theoretical and experimental yields of ethanol.

To perform correctly, the tip of the pH sensor must be oriented downward and submerged in the liquid analyzed. Thus, 14 L of distilled water was added to the reactor at an early stage to facilitate submersion of the pH sensor and accurate pH measurements. The volume was ultimately increased to 60 L with 3 M sulfuric acid to adjust the pH to 5 after the pretreated, washed biomass was

Table 3. Summary of the SSF process

Parameter of SSF	Value
Initial volume of water added [L]	14.0
Inoculation volume [L]	3.0
Volume of enzymes added [L]	1.5
Wet biomass after pretreatment [kg]	40.3
Acid (3 M H_2SO_4) added [L]	2.5
Working volume [L]	60
Theoretical ethanol concentration [g/L] (excluding ethanol from glucose of the inoculation and enzyme stock solution)	66.0
Final ethanol concentration [g/L]	64.0
Ethanol concentration [g/L] (excluding ethanol from glucose of the inoculation and enzyme stock solution)	59.3
Yield [%]	89.9
Productivity [g/L·h] (Approach time to 95% of maximum ethanol conc.)	1.21
Total conversion ratio [%] (Dry biomass to ethanol)	0.14



[kg]

Component	1	2	3	4	5	6	7	8	9	10	11	12	13
Total flow	**25.9**	**30.9**	**11.4**	**45.4**	**65.5**	**131.8**	**40.8**	**14.0**	**1.5**	**2.5**	**3.0**	**3.5**	**58.3**
Cellulose	9.0		2.2	6.8			6.8						
Hemicellulose	4.2		2.0	2.2			2.2						2.2
Lignin	5.9		3.8	2.1			2.1						2.1
Others	4.2		3.3	0.9			1.9				0.1		4.8
Water	2.6	30.0		32.6	65.5	129.6	27.4	14.0		1.8	2.6		45.5
Glucose									0.3		0.3		
NaOH		0.9		0.9		2.2	0.5						
H2SO4										0.7			
Enzymes									1.2				
Ethanol													3.7
CO2												3.5	

Fig. 4. Material balance of bioethanol production with the continuous integrated process.

1. The component analysis of raw biomass
2. 0.72 M NaOH solution 30 L
3. Component of raw biomass [kg] $\times\left(1-\frac{\text{Solid ratio after pretreatment [\%]}\times\text{component of pretreated biomass [\%]}}{\text{component of raw biomass [\%]}}\right)$
4. 4=(1+2)−3
5. Liquid volume after 1st separation=added washing water=2.7 L/h
6. Total volume of recycling pretreatment solution
7. Pretreated biomass (moisture 63%) feeding rate=1.7 kg/h, assuming no loss in the CHESAF
8. Initial water volume for protecting pH sensor
9. Thirty FPU/g cellulose (Cellic® CTec2) and 15% Cellic® HTec2
10. Amount of 3 M H_2SO_4 for adjusting pH
11. Inoculation (~5% total volume)
12. Inverse operation from produced ethanol mass
13. Final production of bioethanol using integrated process

Table 4. The previous study of bioethanol production from various biomass

Raw biomass	Pretreatment methods	Maximum ethanol conc. [g/L]	Maximum ethanol yield [%]	Source
Reed	H_3PO_4-acetone	50.5	98.7	Hui et al., 2009 [28]
Corn stover	Wet oxidation	52	83	Varga et al., 2004 [29]
Wheat straw	Dilute sulfuric acid	57	80	Mohagheghi et al., 1992 [30]
Miscanthus	NaOH pretreatment	59.20	83.9	Han et al., 2011 [31]
Miscanthus	NaOH pretreatment	59.30	89.9	This study

put in with enzymes and yeast. In theory, 66.0 g/L of ethanol from pretreated biomass with a concentration at 23.1% can be generated. Although the final concentration of ethanol measured was 64.0 g/L, it should be noted that 59.3 g/L was actually produced in the reactor (when ethanol derived from glucose of enzyme stock solution and inoculation is excluded), marking an 89.9% yield. This result suggests that the production of cellulosic bioethanol could be commercially feasible, as the yield rate showed similar or higher levels than that of starch-based ethanol production [27]. Table 4 shows the previous study of bioethanol production with SSF process from various biomass. Compared with the previous study, this study shows enhanced results.

4. Material Balance of Bioethanol Production with the Continuous Integrated Process

Pretreating *Miscanthus* with NaOH solution facilitated the efficient production of bioethanol. Based on this outcome, a material balance was achieved. Fig. 4 shows a process flowchart and describes the material balance of bioethanol production from *Miscanthus* cellulosic biomass, using the integrated process. As shown in Fig. 3, 3.7 kg of ethanol and 3.5 kg of CO_2 were made from 25.9 kg of milled *Miscanthus* (containing 10% moisture), which equates to a unit production base of 0.14. Stated differently, at this rate, 140 kg of ethanol can be produced from 1 ton of *Miscanthus*.

Table 5 summarizes the effects of reusing the pretreatment solution. The amounts of water and NaOH needed to prepare pretreatment solution for experiments performed in this study were 94.8 L and 2.80 kg, respectively. Without reusing the pretreatment solution, the amount of water and NaOH required would have been 194.4 L and 3.8 kg, respectively. Thus, by reusing the pretreatment solution, 99.6 L of water and 1.0 kg of NaOH were saved in the process. This process also offers the advantage that wastewater is reduced. If the pretreatment solution were not reused, then the production of ethanol would be increased slightly; however, when considering the costs savings associated with reduced pretreatment solution and wastewater treatment, reusing the pretreatment solution is overall more effective and economical. This study shows promising effects, despite its being performed at the bench scale. Our results suggest that the viable commercial production of bioethanol from cellulosic biomass is just around the corner.

Table 5. Effects of reusing the pretreatment solution

Reuse	Process water [L]	Pretreatment solvent [kg]	Wastewater [L]
No	[a]194.4	[c]3.8	[e]158.4
Yes	[b]94.8	[d]2.8	None or reusable

[a]Pretreatment solution: 5.4 L/h * 24 h=129.6 L; Wash water: 2.7 L/h * 24 h=64.8 L

[b]Wash water: 2.7 L/h * 24 h=64.8 L; Initial pretreatment solution: 30 L

[c]0.72 M NaOH solution 129.6 L (purity 0.97)

[d]Refer to Table 2

[e]Thirty-six litres of process water was added to the SSF tank with pretreated biomass

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REFERENCES

1. A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney and C. A. Eckert, *Science*, **311**, 484 (2006).
2. A. J. Ragauskas, M. Nagy, D. H. Kim, C. A. Eckert, J. P. Hallett and C. L. Liotta, *Ind. Biotechnol.*, **2**, 55 (2006).
3. A. Sorensen, P. J. Teller, T. Hilstrom and B. K. Ahring, *Bioresour. Technol.*, **99**, 6602 (2008).
4. T. de Vrije, G. G. de Haas, G. B. Tan, E. R. P. Keijsers and P. A. M. Claassen, *Int. J. Hydrogen Energy,* **27**, 1381 (2002).
5. J. Eitzinger and C. Kossler, *Theor. Appl. Climatol.*, **72**, 245 (2002).
6. M. Himken, J. Lammet, D. Neukirchen, U. Czypionka-Kause and H.-W. Olfs, *Plant Soil.*, **189**, 117 (1997).
7. I. Lewandowski, J. C. Clifton-Brown, B. Andersson, G. Brasch, D. G. Chrsitian, U. Jorgensen, M. B. Jones, A. B. Riche, K. U. Schwarz, K. Tayebi and F. Teixeira, *Agron J.*, **95**, 1274 (2003).
8. P. C. Badger, *Ethanol from cellulose: A general review in Trends in new crops and new uses (Ed.: Janick J, Whipkey A),* ASHS Publishers, Alexandria VA (2002).
9. A. O. Converse, H. Ooshima and D. S. Burns, *Appl. Biochem. Biotechnol.*, **24-25**, 67 (1990).
10. N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple and M. Ladisch, *Bioresour. Technol.*, **96**, 673 (2005).
11. S. J. Patel, R. Onkarappa and K. S. Shobha, *Electron. J. Environ. Agr. Food Chem.*, **6**, 1921 (2007).
12. P. Kumar, D. M. Barrett, M. J. Delwiche and P. Stroeve, *Ind. Eng. Chem. Res.*, **48**, 3713 (2009).
13. M. J. Diaz, C. Cara, E. Ruiz, I. Romero, M. Moya and E. Castro, *Bioresour. Technol.*, **101**(7), 2428 (2010).

14. T. A. Hsu, *Pretreatment of biomass in Handbook on bioethanol production and utilization (Applied Energy Technology Series) (Ed.: Wyman CE)* Taylor & Francis, Washington DC (2010).
15. B. E. Dale, L. L. Henk and M. Shiang, *Dev. Ind. Microb.*, **26**, 223 (1984).
16. H. H. Yoon, *Korean J. Chem. Eng.*, **15**(6), 631 (1998).
17. H. H. Brownell, E. K. C. Yu and J. N. Saddler, *Biotechnol. Bioeng.*, **28**, 792 (1986).
18. B. Klinpratoom, A. Ontanee and C. Ruangviriyachai, *Korean J. Chem. Eng.*, **32**(3), 413 (2015).
19. M. Han, Y. Kim, S. W. Kim and G. W. Choi, *J. Chem. Technol. Biotechnol.*, **86**(12), 1527 (2011).
20. S. H. Lee, Y. Teramoto, N. Tanaka and T. Endo, *Bioresour. Technol.*, **100**(1), 275 (2009).
21. S. H. Lee, S. Inoue, Y. Teramoto, N. Tanaka and T. Endo, *Bioresour. Technol.*, **101**(24), 9645 (2010).
22. M. Han, K. E. Kang, Y. Kim and G. W. Choi, *Process Biochem.*, **48**(3), 488 (2013).
23. B. Lamsal, K. Brijwani and S. Alavi, *Biomass Bioenergy*, **34**(12), 1703 (2010).
24. P. W. Madson, Ethanol distillation: The fundamentals, in The alcohol Textbook, Ed. Ingledew WM, Kelsall DR, Austin GD and Kluspies C. Nottingham University Publishers, Nottingham, 292 (2009).
25. M. Han, S. E. Moon and G. W. Choi, *Bioprocess Biosyst. Eng.*, **37**(11), 2205 (2014).
26. National Renewable Energy Laboratory, Standard Biomass Analytical Procedures, http://www.nrel.gov/biomass/analytical_procedures.html.
27. G. W. Choi, S. E. Moon, H. W. Kang, J. Min and B. W. Chung, *J. Chem. Technol. Biotechnol.*, **84**, 547 (2009).
28. L. Hui, N. Kim, J. Min, J. W. Kang and H. N. Janog, *Bioresour. Technol.*, **100**, 3248 (2009).
29. E. Varga, H. B. Klinke, K. Reczey and A. B. Thomsen, *Biotechnol. Bioeng.*, **88**, 517 (2004).
30. A. Mohagheghi, M. Tucker, K. Grohmann and C. Wyman, *Appl. Biochem. Biotechnol.*, **33**, 67 (1992).
31. M. Han, Y. Kim, B. Koo and G. W. Choi, *Bioresources*, **6**(2), 1939 (2011).