Korean J. Chem. Eng., 34(2), 346-352 (2017)
DOI: 10.1007/s11814-016-0320-2

pISSN: 0256-1115
eISSN: 1975-7220

Pretreatment of *Helianthus tuberosus* residue by flow-through process for production of fermentable sugar

Yong Cheol Park and Jun Seok Kim†

Department of Chemical Engineering, Kyonggi University, Suwon 16227, Korea
(*Received 27 April 2016 • accepted 9 September 2016*)

Abstract−The pretreatment of *Helianthus tuberosus* residue was studied for fermentable sugar production. The pretreatment was performed by varying the temperature, type of chemical solution, and concentration. Two different catalytic pretreatments using sulfuric acid and aqueous ammonia were operated and compared in a flow-through column reactor system. The flow-through process was required to increase the sugar production yield of biomass. To selectively remove the lignin of biomass and achieve fractionation of hemicellulose in the liquid phase to produce pentose, the flow-through process could be controlled by the pretreatment conditions. Furthermore, the remaining solid underwent enzymatic hydrolysis for hexose production. The mass balances of biomass pretreated with aqueous ammonia and sulfuric acid solution were compared in terms of production of fermentable sugars. The glucose recovery compared to the initial biomass was 71.2% in the pretreatment using aqueous ammonia at 170 °C, and pretreatment using sulfuric acid solution at 150 °C was 52.3%.

Keywords: Cellulosic Biomass, Pretreatment, Flow-through, *Helianthus tuberosus* Residue, Aqueous Ammonia, Sulfuric Acid

INTRODUCTION

Lignocellulosic materials have attracted much attention, as they offer a renewable source of fermentable sugars for producing biofuels [1]. Lignocellulosic biomass is a complex polymeric material comprised of cellulose, hemicellulose, lignin, and other components. Many factors affect the biological conversion of lignocellulosic biomass, including chemical and physical barriers that inhibit its enzymatic hydrolysis. The presence of lignin, a hydrophobic heteropolymer of three monomers, has been proven to be a major hindrance for enzymatic reactions. Lowering the lignin content is desirable during pretreatment to enhance enzymatic hydrolysis [2,3].

The major purpose of aqueous ammonia pretreatment is to remove lignin. Aqueous ammonia pretreatment can remove 60% of lignin from cellulosic biomass while achieving 70% enzymatic digestibility. In addition, studies have shown that ammonia can selectively react with lignin bonds, as well as ester and ether bonds, especially ether bonds, causing the selective removal of lignin in biomass [4-6]. Acid hydrolysis is one of the most promising pretreatment methods. Dilute sulfuric acid pretreatment has been studied for many types of lignocellulosic biomass. It has resulted in high recovery of the hemicellulose in the pretreatment liquid and in a solid cellulose fraction [7,8]. Both concentrated and diluted acids have been used to pretreat lignocellulosic biomass. Acid pretreatment can be applied to solubilize partial hemicelluloses from lignocellulosic biomass. However, the analysis of this propensity is fundamentally the result of a batch reaction. The batch reaction process should be used for analysis after removing the reaction conditions (temperature, pressure, etc.). After the reaction, it cannot show the effect of condensation. Therefore, a flow-through process is required. However, the concentrated acid pretreatments likely lead to severe cellulose degradation, high inhibitor concentrations, and serious equipment corrosion [9,10]. To avoid degrading cellulose/hemicellulose and thus forming inhibitors, a flow-through reactor for pretreatment of lignocellulosic biomass at high temperatures and pressure (300 psig) is required. A flow-through column reactor performs reasonably in this regard because it is a reactor packed with biomass, which allows operations with a high solid/liquid ratio, reaches the work temperature quickly, and may enable interpretation of the clear effect of fractionation in the desired time [11-13]. When pretreatment is performed using a batch process, both the pretreatment solution and pretreated biomass remain together on the reactor, structurally. Therefore, it is disadvantageous that both the fractionated component as the liquid phase and pretreated biomass are combined once more. However, because the pretreatment solution can be stored in the reservoir tank without leaving the reactor by controlling the reaction time, the flow-through process can be reduced to combine both the fractionated component and pretreated biomass, structurally. Therefore, it is possible to analyze clearly the removal effect of inhibitors of enzymatic hydrolysis according to the pretreatment conditions.

In this study, we sought to increase glucan yield by removing the lignin with aqueous ammonia while keeping enough cellulose and hemicellulose intact. The acid pretreatment involved using dilute sulfuric acid to solubilize hemicellulose. The ammonia-pretreated solids contained mostly glucan with low lignin content, while the sulfuric-acid-pretreated solids contained low xylan content. Both pretreatment methods were evaluated to determine the

†To whom correspondence should be addressed.
E-mail: jskim84@kyonggi.ac.kr

effects on biomass composition and the production of fermentable sugars.

MATERIALS AND METHODS

1. Materials

Helianthus tuberosus residue was provided by the Korea Research Institute of Bioscience and Biotechnology (KRIBB). It was milled below 50 mesh. 10-20 wt% of aqueous ammonia (Duksan, CAS No. 1336-21-6) and 0.5-2.0 wt% of sulfuric acid (Duksan, CAS No. 7664-93-9) were used. For enzymatic hydrolysis, the enzymes were Celluclast 1.5L (CAS No. 9012-54-8) and Novozyme-188 (CAS No. 9001-22-3) [16].

2. Flow-through Pretreatment (Acid and Alkaline Pretreatment)

The *Helianthus tuberosus* residue was pretreated by using a flow-through column reactor. The pretreatment reaction was performed at 150-190 °C. The flow rate of the liquid was 4 mL/min for 40 min in the flow-through process. The reaction time was selected according to a previous work based on the solid/liquid ratio, which was 1/10. The system consisted of a stock solution reservoir, pump, temperature-programmable oven, SS-316 column reactor (3 cm internal diameter×19.4 cm length, internal volume of 137 cm^3), and liquid-holding tank. The reactor was operated in flow-through mode, in which the liquid flowed through the reactor column packed with biomass. The reactor system was pressurized with nitrogen at 2.3 MPa to prevent flash evaporation. In a typical flow-through experiment, 35 g of biomass was packed into the reactor. The reaction was initiated by raising the reactor temperature in a forced-air convection oven. Approximately 15 min of preheating was required to reach the desired temperature. The reaction time was counted after the desired temperature was attained. All of the flow-through experiments were run in duplicate [14-16].

3. Enzymatic Digestion

The pretreated *Helianthus tuberosus* residue was hydrolyzed in Erlenmeyer flasks. The enzymatic digestions were performed in a 0.1 M citrate buffer solution (pH 4.8) shaken at 180 rpm for 72 h. The conditions of enzymatic digestion were a substrate concentration of 4 wt%, temperature of 50 °C, and enzyme loading of 60 FPU (filter paper unit)/mL of substrate and 120 CBU/mL (CBU: cellobiose unit) of substrate [15-19].

$$\text{Enzymatic digestibility [\%]} = \frac{\text{Amount of glucose released (g)} \times 0.9}{\text{Total initial glucan (g)}} \times 100$$

4. Analytical Methods

The compositions of sugars and acid-insoluble lignin (AIL) were determined according to the National Renewable Energy Laboratory (NREL) Standard Biomass Analytical Procedures [19]. The compositions of the hydrolysates from the enzymatic digestion were determined using high-performance liquid chromatography (HPLC). The HPLC system consisted of a Bio-rad Aminex HPX-87H column and a refractive index detector. The mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 mL/min and at 60 °C. Prior to injection into the HPLC apparatus, all samples were centrifuged at 15,000 rpm for 10 min and filtered through 0.2 μm syringe filters [15-18].

RESULTS AND DISCUSSION

1. Biomass Composition

Helianthus tuberosus residue was used in the study as lignocellulosic biomass. The raw *Helianthus tuberosus* residue was composed of 41.7% cellulose, 19.2% hemicellulose, and 20.9% acid-insoluble lignin (AIL).

2. Pretreatment Using Aqueous Ammonia and Sulfuric Acid

We performed the pretreatment of *Helianthus tuberosus* residue by using a flow-through process using aqueous ammonia and sulfuric acid at temperatures varying from 150 to 190 °C. Before the delignification reaction of aqueous ammonia, the biomass was pretreated by a flow-through process. Xylose was recovered as a liquid phase using sulfuric acid by the flow-through process, and was measured in the solid remaining for the pretreated biomass. Changes of each component after pretreatment were due to the yield of produced sugar in enzymatic hydrolysis. The amounts of solid remaining are shown in Table 1. The difference in the amount of solid remaining in biomass pretreated with aqueous ammonia was too small. In this case, the amount of solid remaining was 55-56%. The amount of solid remaining in biomass after pretreatment with sulfuric acid was 32-60%. The amount of solid remaining had a large dependence on the temperature. It was confirmed that the cellulose and hemicellulose in the pretreatment process using aqueous ammonia were conserved, and there was little to no difference in the amount lost by the reaction. In the case of biomass pretreated using sulfuric acid solution, the hemicellulose was mostly in the liquid, as is characteristic of pretreatment using an acid solution. It was confirmed that a large amount of cellulose was fractionated into the liquid phase when reacted at 190 °C.

The components of biomass pretreated under various pretreat-

Table 1. Solid remaining of pretreatment biomass

Solutions	Concentration (wt%)	Temperature (°C)	Solid remaining (%)
Aqueous ammonia	10	150	68.0
		170	62.4
		190	59.6
	15	150	67.8
		170	61.1
		190	60.4
	20	150	64.5
		170	57.5
		190	54.7
Sulfuric acid	0.5	150	58.4
		170	50.9
		190	36.9
	1.0	150	59.9
		170	51.6
		190	34.8
	2.0	150	55.8
		170	44.0
		190	31.6

Table 2. Component analysis of biomass by pretreatment conditions

Pretreatment conditions			Components (%)				
Solutions	Conc. (wt%)	Temp. (°C)	Cellulose	Hemi-cellulose	Lignin	Others	Total
Aqueous ammonia	10	150	36.8	12.4	13.5	5.4	68.0
		170	37.3	9.1	12.0	3.9	62.4
		190	38.9	9.7	10.9	0.1	59.6
	15	150	38.3	12.1	12.6	4.8	67.8
		170	36.3	9.2	9.6	6.0	61.1
		190	37.0	8.5	9.0	5.9	60.4
	20	150	35.8	11.1	11.2	6.2	64.5
		170	35.4	9.2	8.1	4.7	57.5
		190	33.5	8.0	7.1	6.0	54.7
Sulfuric acid	0.5	150	34.8	0.0	17.1	6.6	58.4
		170	30.0	0.0	16.2	4.7	50.9
		190	16.7	0.0	17.6	2.5	36.9
	1.0	150	32.5	0.0	17.2	10.2	59.9
		170	26.7	0.0	17.7	7.3	51.6
		190	8.0	0.0	17.4	9.4	34.8
	2.0	150	29.6	0.0	16.8	9.4	55.8
		170	24.1	0.0	15.6	4.3	44.0
		190	2.5	0.0	16.2	12.9	31.6
Raw biomass			41.7	19.2	20.9	18.3	100.0

Table 3. Sugar recovery and lignin removal after pretreatment process

Pretreatment conditions			Cellulose recovery (%)	Hemi-cellulose recovery (%)	Lignin removal (%)
Temperature (°C)	Solutions	Concentration (wt%)			
150	Aqueous ammonia	10	88.3	64.4	35.4
		15	91.9	63.1	39.6
		20	86.0	58.1	46.1
	Sulfuric acid	0.5	83.4	0.0	18.1
		1.0	78.0	0.0	17.6
		2.0	71.0	0.0	19.4
170	Aqueous ammonia	10	89.5	47.4	42.3
		15	87.0	47.8	53.8
		20	85.0	48.2	61.0
	Sulfuric acid	0.5	71.9	0.0	22.2
		1.0	64.1	0.0	15.2
		2.0	57.8	0.0	25.1
190	Aqueous ammonia	10	93.3	50.8	47.6
		15	88.8	44.6	57.0
		20	80.5	41.9	65.8
	Sulfuric acid	0.5	40.1	0.0	15.6
		1.0	19.3	0.0	16.5
		2.0	6.0	0.0	22.1

ment conditions are shown in Table 2. Table 3 shows the results of pretreatment using the flow-through process with each solution at 150-190 °C. The cellulose recovery was approximately 90% when pretreated with 10, 15, and 20 wt% aqueous ammonia. The hemi-cellulose recovery was approximately 64% when pretreated with 10 and 15 wt% aqueous ammonia and approximately 58% when pretreated with 20 wt% aqueous ammonia. The delignification of pretreated biomass was 35-46%. Generally, the values were higher

at higher temperatures. In the pretreatment with sulfuric acid, most of the hemicellulose was removed and delignification was approximately 18%. It was shown that the results of pretreatment were not significantly affected by the concentrations of the aqueous ammonia and sulfuric acid solutions at 150 °C. According to Table 3, delignification evidently increased when the temperature was higher than 170 °C. When the biomass was pretreated using sulfuric acid, the amount of solid remaining for cellulose decreased significantly at increasing temperatures. In particular, the cellulose was only 6% at 190 °C.

3. Enzymatic Digestibility

The pretreated *Helianthus tuberosus* residue underwent enzymatic hydrolysis. The pretreated biomass was compared with raw biomass. In the case of the enzymatic hydrolysis of raw *Helianthus tuberosus* residue, as the concentration and conversion yield of glucose were 2.4 g/L and 14.2%, respectively, enzymatic hydrolysis did not have a strong positive effect. According to Table 4, the enzymatic hydrolysis of *Helianthus tuberosus* residue, which underwent pretreatment with aqueous ammonia, showed a glucose conversion yield higher than 60%; the glucose concentration was 15.6 g/L. This concentration represented an approximately six-fold increase compared to that of raw biomass. It was shown that the removal of lignin as an inhibition factor had significantly affected glucose production. When glucose conversion was increased through optimization of pretreatment conditions, higher concentrations of glucose seemed to be produced. In the case of biomass pretreated with 20 wt% aqueous ammonia, the glucose concentration was 21.2 g/L and the glucose conversion yield was 86.3% at a high reaction temperature of 190 °C. On the other hand, in the case of biomass pretreated with 0.5 wt% sulfuric acid at 150 and 170 °C, a glucose concentration of 15 g/L was obtained and the glucose conversion yield was approximately 64%. These results indicated that the removal of hemicellulose had also led to increases in the glucose conversion yield. Although the glucose conversion yield was higher than 90% at a high temperature of 190 °C, the glucose concentration was significantly lower than in the pretreated biomass at other temperatures. The glucose concentration was approximately 4%.

As a result, it was confirmed that when performing pretreatment using aqueous ammonia, changes in the reaction temperature had no significant effect on the enzymatic hydrolysis. There were small differences with changes in temperature, but all values were determined to be approximately within the error range. The values for biomass pretreated using aqueous ammonia at a high concentration were 40% higher than those of biomass pretreated at low concentration. When the concentration of the pretreatment solution was high, delignification, which loosens the structure of biomass, increased. When reacted with enzymes, it was determined that the surface area for enzyme reaction was wide. Similarly, biomass underwent pretreatment with sulfuric acid though delignification was low. The hemicellulose was fractionated mostly into the liquid phase, and the pretreated biomass retained its components except for hemicellulose in the solid phase. In addition, much of the cellulose could be removed depending on the reaction temperature and concentration of the sulfuric acid solution. The surface area available to make contact with the enzyme increased because the process was able to create a considerable amount of empty space in the structure while removing the cellulose and hemicellulose from biomass. The biomass that reacted at a high reaction temperature and with

Table 4. The concentration and conversion yield of sugar after enzymatic hydrolysis for pretreated biomass

Pretreatment conditions			Conversion (%)		Concentration (g/L)	
Solution	Concentration (wt%)	Temperature (°C)	Glucose	Xylose	Glucose	Xylose
Aqueous ammonia	10	150	63.6	81.0	13.8	5.9
		170	62.8	78.6	15.0	4.6
		190	59.8	67.4	15.6	4.3
	15	150	68.4	85.1	15.5	6.1
		170	78.9	90.6	18.8	5.4
		190	68.0	74.6	16.6	4.2
	20	150	77.1	67.4	17.1	4.7
		170	83.8	68.1	20.6	4.4
		190	86.3	73.2	21.2	4.3
Sulfuric acid	0.5	150	62.7	-	14.9	-
		170	63.7	-	15.0	-
		190	87.8	-	15.9	-
	1	150	63.8	-	13.8	-
		170	69.8	-	14.4	-
		190	99.8	-	9.6	-
	2	150	51.1	-	10.8	-
		170	59.7	-	13.1	-
		190	99.3	-	3.7	-
Raw biomass			14.2	8.3	2.4	0.6

a sulfuric acid solution at a high concentration was fractionated to a relatively large extent into the cellulose in the liquid phase. The amount of solid remaining from biomass pretreated using sulfuric acid was substantially low. Although the glucose conversion was higher, because of a lower amount of glucose, the glucose was produced in a low concentration. Therefore, it has been confirmed that a higher glucose conversion as a result of enzymatic hydrolysis was more important than the glucose concentration.

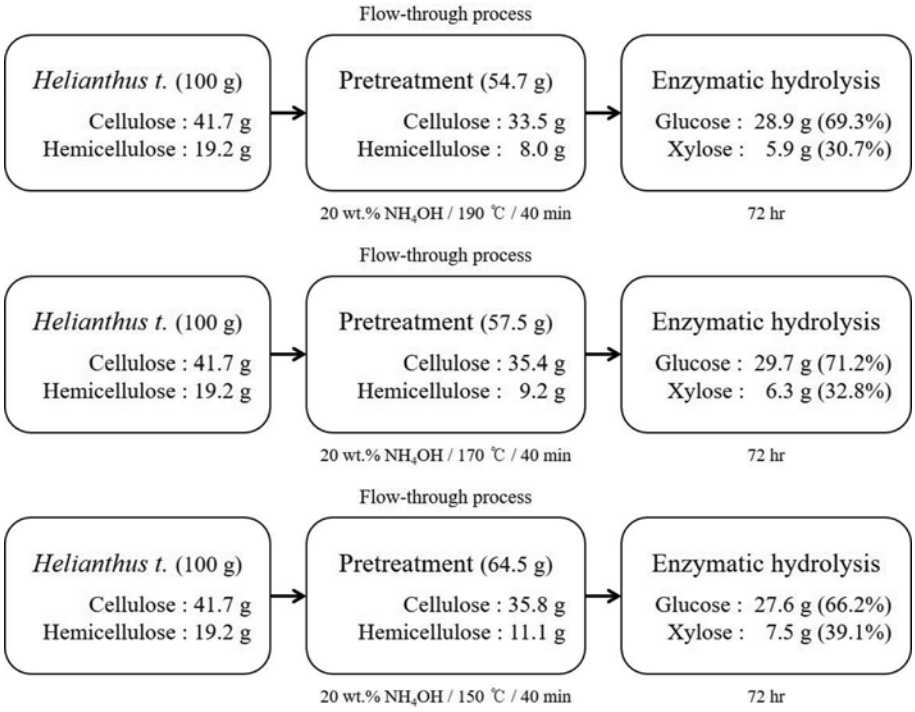

Fig. 1. The mass balance of sugars after enzymatic hydrolysis by pretreated biomass using 20 wt% aqueous ammonia at 40 min depending on the conditions of various temperature.

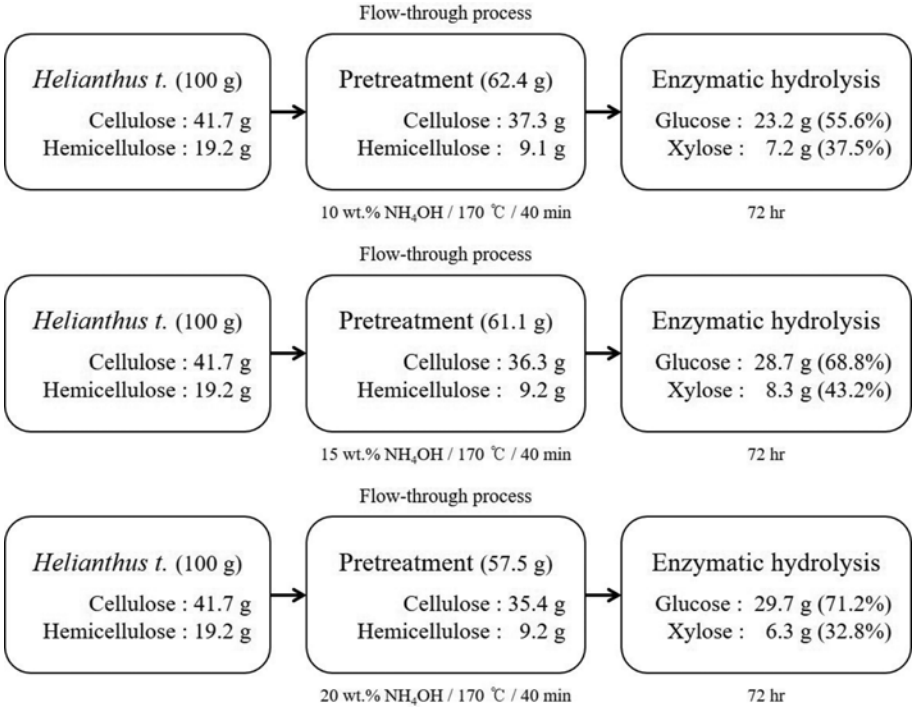

Fig. 2. The mass balance of sugars after enzymatic hydrolysis by pretreated biomass for 40 min at 170 °C depending on the conditions of various aqueous ammonia concentration.

4. Mass Balance of Pretreated Biomass

The mass balances of biomass pretreated with aqueous ammonia and sulfuric acid solution were compared in terms of sugar concentration and conversion yield. The mass balances of sugars after enzymatic hydrolysis of biomass pretreated using 20 wt% aqueous ammonia for 40 min at various temperatures are shown in Fig. 1. The glucose recovery compared to the initial biomass for the biomass pretreated with aqueous ammonia at 170 °C was 71.2%. The

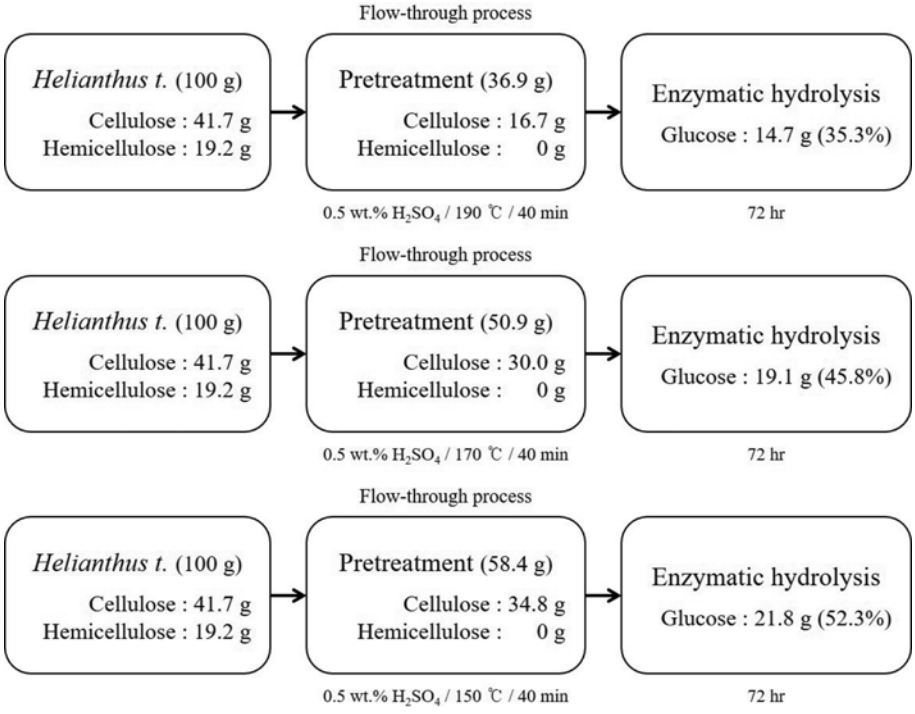

Fig. 3. The mass balance of sugars after enzymatic hydrolysis by pretreated biomass using 0.5 wt% sulfuric acid solution at 40 min depending on the conditions of various temperature.

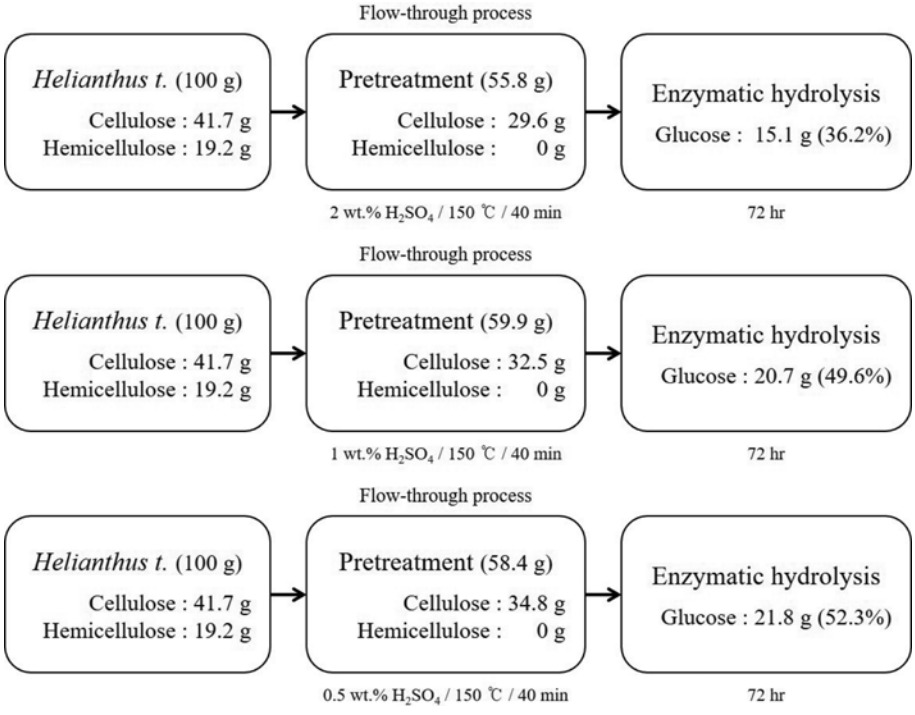

Fig. 4. The mass balance of sugars after enzymatic hydrolysis by pretreated biomass for 40 min at 150 °C depending on the conditions of various sulfuric acid concentration.

xylose recovery was 39.1% in the pretreatment process carried out at 150 °C. The glucose conversions after the pretreatment process using aqueous ammonia at 190 °C were higher than those at other temperatures. The glucose recovery compared to the initial biomass was lower than those at other temperatures. Because of the considerable sugar loss at high temperatures, it was determined that the amount of solid remaining at 190 °C was lower than that at below 170 °C. Fig. 2 shows the mass balances of sugars after enzymatic hydrolysis of biomass pretreated for 40 min at 170 °C at various aqueous ammonia concentrations. The amount of remaining glucan was relatively high when the concentration of aqueous ammonia was low. The recovery of sugar upon pretreatment with 20 wt% aqueous ammonia was higher than that after pretreatment with 10 wt% aqueous ammonia. It was determined that the biomass structure was lost because the surface area was enlarged to react with the enzyme owing to the removal of much of the lignin by high-concentration aqueous ammonia.

The mass balances of sugars after enzymatic hydrolysis of biomass pretreated using sulfuric acid solution are shown in Figs. 3 and 4. The recovery of glucose of the biomass pretreated using a 0.5 wt% sulfuric acid solution compared to the initial biomass was 52.3% at 150 °C. The glucose conversion yields with pretreatment processes using sulfuric acid solution were much higher at higher temperatures, similar to the process using aqueous ammonia. The total balance was not high because of the low amount of glucose due to the loss of solid. The results showed that it was not necessary to apply the process at high temperatures to increase the recovery to some extent while ensuring that the solid remained. In addition, it was confirmed that higher concentrations of sulfuric acid increased the loss of solids. To increase sugar production, it was best to reduce the loss of sugar by lowering the reaction temperature. The production of sugar by enzymatic hydrolysis was better correlated with lignin removal by using aqueous ammonia than with fractionation of hemicellulose by using sulfuric acid.

CONCLUSIONS

Helianthus tuberosus residue was pretreated by using a flow-through column reactor. The pretreatment used aqueous ammonia and sulfuric acid solution. The amount of solid remaining was influenced by the temperature, and the pretreatment using sulfuric acid was compared with that using aqueous ammonia.

The pretreated *Helianthus tuberosus* residue underwent enzymatic hydrolysis. The concentration and conversion yield were almost unaffected by ammonia concentration and reaction temperature. The glucose recovery compared to the initial biomass was 71.2% in the pretreatment using aqueous ammonia and 52.3% in that using sulfuric acid solution. The production of fermentable sugar through enzymatic hydrolysis was better correlated with lignin removal by using aqueous ammonia than with the removal of hemicellulose by using sulfuric acid solution.

The flow-through process is required to increase the sugar production yield of biomass. The flow-through process could control the pretreatment conditions to enable selective fractionation of the components of biomass. Therefore, it is possible to determine pretreatment conditions for efficient glucose production.

ACKNOWLEDGEMENTS

This work was supported by the New & Renewable Energy Core Technology Program of the Korea Institute of Energy Technology Evaluation and Planning (KETEP), granted financial resource from the Ministry of Trade, Industry & Energy, Republic of Korea (No. 20153010091990).

REFERENCES

1. P. M. Abdul, J. M. Jahim, S. Harun, M. Markom, N. A. Lutpi, O. Hassan, V. Balan, B. E. Dale and M. T. M. Nor, *Bioresour. Technol.*, **211**, 200 (2016).
2. T. H. Kim, Wiley, New York, USA, 91 (2013).
3. J. S. Kim, Y. Y. Lee and T. H. Kim, *Bioresour. Technol.*, **199**, 42 (2016).
4. D. Wanga, J. Xic, P. Aia, L. Yud, H. Zhaia, S. Yana and Y. Zhang, *Bioresour. Technol.*, **207**, 52 (2016).
5. E. Jurado, I. V. Skiadas and H. N. Gavala, *Appl. Energy*, **109**, 104 (2013).
6. A. H. Gao, M. V. Bule, D. D. Laskar and S. Chen, *J. Agricultural Food Chem.*, **60**, 8632 (2012).
7. F. Hu and A. Ragauskas, *Bioenergy Res.*, **5**, 1043 (2012).
8. L. J. Jönsson and C. Martín, *Bioresour. Technol.*, **199**, 103 (2016).
9. P. Alvira, E. Tomás-Pejó, M. Ballesteros and M. J. Negro, *Bioresour. Technol.*, **101**, 4851 (2010).
10. S. Sun, S. Sun, X. Cao and R. Sun, *Bioresour. Technol.*, **199**, 49 (2016).
11. R. Terán-Hilares, A. L. Reséndiz, R. T. Martínez, S. S. Silva and J. C. Santos, *Bioresour. Technol.*, **203**, 42 (2016).
12. P. Reddy, P. Lekha, W. Reynolds and C. Kirsch, *Bioresour. Technol.*, **183**, 259 (2015).
13. L. H. D. S. Martins, S. C. Rabelo and A. C. D. Costa, *Bioresour. Technol.*, **191**, 312 (2015).
14. T. H. Kim, J. S. Kim, C. Sunwoo and Y. Y. Lee, *Bioresour. Technol.*, **90**, 39 (2003).
15. K. S. Kim and J. S. Kim, *Korean Chem. Eng. Res.*, **48**, 704 (2010).
16. Y. C. Park and J. S. Kim, *Energy*, **47**, 31 (2012).
17. Y. C. Park, J. W. Kim and J. S. Kim, *Korean Chem. Eng. Res.*, **49**, 292 (2011).
18. Y. C. Park and J. S. Kim, *Korean Chem. Eng. Res.*, **49**, 470 (2011).
19. *Chemical analysis and testing laboratory analytical procedures*, National Renewable Energy Laboratory (1996).