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Comparison of bioethanol production of simultaneous saccharification & fermentation and separation hydrolysis & fermentation from cellulose-rich barley straw

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Abstract−Cellulose rich barley straw, which has a glucan content of 62.5%, followed by dilute acid pretreatment, was converted to bioethanol by simultaneous saccharification and fermentation (SSF). The optimum fractionation conditions for barley straw were an acid concentration of 1% (w/v), a reaction temperature of 158 °C and a reaction time of 15 min. The maximum saccharification of glucan in the fractionated barley straw was 70.8% in 72 h at 60 FPU/g-glucan, while the maximum digestibility of the untreated straw was only 18.9%. With 6% content WIS (water insoluble solid) for the fractionated barley straw, 70.5 and 83.2% of the saccharification yield were in SHF and SSF (representing with glucose equivalent), respectively, and a final ethanol concentration of 18.46 g/L was obtained under the optimized SSF conditions: 34 °C with 15 FPU/g-glucan enzyme loading and 1 g dry yeast cells/L. The results demonstrate that the SSF process is more effective than SHF for bioethanol production by around 18%.

Key words: Barley Straw, Bioethanol, Fractionation, Simultaneous Saccharification Fermentation (SSF), Pretreatment

INTRODUCTION

Bioethanol produced from lignocellulosic resources is beneficial as it reduces the consumption of petroleum-based fuels that accelerate warming of the world *via* climate change. Bioethanol can be produced from different kinds of raw materials such as starch, sugar, or cellulose-containing biomass. The current focus is on bioethanol production from crops as well as from highly abundant agricultural residues [1]. Agricultural residues, such as rice straw, rape straw, corn stover, and barley straw, have been used for feeding ruminants. However, the practical use of the aforementioned agricultural residues is not very high: only 20% of residues are used as feeding resources and then the remnant is consumed non-productively, for example, through incineration, *etc*., because of the high dependency of a preference for feeding and a lack of effective preservation know-how. As, at present, only a very small fraction of them are used for renewable energy, the utilization potential of agricultural residues should be considered seriously, and it is essential that the energy stored within biomass be effectively utilized as a carbon-neutral energy source [2]. One major variable that must be considered with bioethanol production is the availability of raw materials. The availability of feedstocks for bioethanol can vary considerably from season to season and depends on geographic locations. The price of raw materials can highly affect the bioethanol production cost. Agricultural residues serve as a cheap and abundant feedstock, which is required to produce fuel bioethanol from renewable resources at reasonable costs.

Barley straw is nowadays being considered as a potential lignocellulosic raw material for fuel-ethanol production, which has the potential to serve as a low cost, renewable organic substance, as it is one of the abundant agricultural residues. About 152 million metric tons of barley is produced annually, corresponding to 53.2 million tons of straw, assuming it takes up around 35% of the total barley crop, and this amount of barley straw can potentially produce 1.5 million kL bioethanol per year, which is the third largest amount from a single biomass feedstock in the world, next to wheat straw and rice straw [3]. It has cellulose, hemicellulose, and lignin contents of about 35-40%, 25-30%, and 12-15% (dry basis), respectively [4,5].

Enzymatic saccharification has the potential to convert the carbohydrates in lignocellulosic biomass to ethanol with high yields and low production costs [6-8]. However, the recalcitrance of the lignocellulosic matrix to enzymatic attack necessitates pretreatment of the biomass in order to enhance the accessibility of the substrate to the enzymes. In the enzymatic saccharification process of barley straw, the matrix structure prevents cellulose from being attacked by enzymes, and the lignin component adsorbs an enzyme, leading to there being fewer effective enzymes for cellulose degradation [9]. Owing to these structural characteristics, pretreatment is an essential step for potentially obtaining fermentable sugars in the saccharification step. The ultimate goal of the fractionation is to remove hemicellulose and/or lignin from the lignocellulosic biomasss in order to increase the cellulose content and disrupt the crystalline structures of the cellulose to enhance the enzymes' accessibility to the cellulose during the saccharification step [10,11].

Thermochemical pretreatment technologies such as dilute acid pretreatment have been widely applied to pretreat lignocellulosic biomass. These processes are able to significantly improve the efficiency of saccharification and fermentation [12-14].

Enzymatic saccharification and fermentation of the pretreated biomass can be carried out in different processes such as separate saccharification and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). SSF process is a promising option since it is carried out in one reactor and product inhibition is minimized, allowing higher solid levels [15]. SSF has been shown to be

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less capital intensive and to result in higher overall ethanol yields, which is highly beneficial for the overall process economy. In addition, SSF reduces processing time, which in turn leads to increases in the production of ethanol. During the SSF process, in which liberated glucose is quickly converted to ethanol by fermenting microorganisms, the inhibition of cellulase is also reduced and this single fermentor process eliminates a portion of the investment cost by reducing the number of fermentors. Moreover, the SSF process reduces the contamination risk due to the presence of ethanol in the medium. However, the performance of the SSF process is limited by the different optimum temperatures for enzymatic saccharification and microbial fermentation. The optimum temperature for cellulases is usually in the range of 40 to 50 °C, while the optimum temperature for ethanol production for the most common ethanologenic yeast, Saccharomyces cerevisiae, is between 30 and 37 °C [16,17]. It is reported that the overall ethanol yield for both softwood [18] and corn stover increases when employing simultaneous saccharification and fermentation (SSF). In addition, higher productivity is achieved in SSF than SHF, which reduces the capital cost [19,20].

The objective of this study was to investigate and compare the feasibility of producing bioethanol for dilute sulfuric acid fractionated barley straw through SSF and SHF. The optimum fractionation condition has already been established in our previous studies. Experiments were also carried out to investigate the extent of enzyme loadings and solid substrate levels (WIS) on the performance of SSF process.

MATERIALS AND METHODS

1. Raw Material

Fractionated barley straw with dilute sulfuric acid was used throughout this study. The raw barley straw was supplied by the Bioenergy Crop Research Center, National Institute of Crop Science, Rural Department Administration (Muan, Jeollanamdo, Korea). The straw was ground to an average size of 14-45 mesh (0.35-1.41 mm) and air-dried for 24 h at 45±5 °C. The moisture content of the milled straw was 5.17% based on total wet biomass weight. The fractionation was carried out under the conditions of 1% (w/v) sulfuric acid concentration for 15 min at a temperature of 158 °C.

2. Chemical Analysis

The compositions of raw barley straw, fractionated straw, hydrolyzate liquor, and ethanol in the fermentation broth were analyzed quantitatively according to NREL Laboratory Analytical Procedures: NREL Standard procedure #002 (Two stage sulfuric acid hydrolysis for determination of carbohydrates), NREL/TP-510-42623 (sugars, byproducts, and degradation products in liquid fraction) and 42627 (insoluble solids in pre-treated biomass) [21-23]. Approx 300 mg (dry basis) was dispensed into separate test tubes. H_2SO_4 (72%, w/w) was added to each of the tubes to create an acid-to-sample ratio of 0.01 mL/mg. The tubes were placed in a reciprocal water bath set at 30 °C for 120 min and stirred every 15 min with dedicated glass stirring rods. Hydrolyzates were diluted to 4% (w/w) H_2SO_4 with distilled water, and a secondary saccharification performed for 60 min at 121 °C. Once secondary saccharification was complete, the serum vials were centrifuged at 2,800 g for 20 min to remove solid particles. The resulting supernatant was decanted and a portion was filtered (Gelman, 0.2 μm pore size). The resulting concentrations of dissolved monomer sugar were determined by chromatography. The Breeze HPLC system (Waters Co., Milford, MA, USA) used for carbohydrate and ethanol concentration measurements had a Bio-Rad Aminex HPX-87H column (300 mm ×7.8 mm) (Bio-Rad Laboratories Inc., Hercules, CA). The column was maintained at 60 °C, with a 5 mM H_2SO_4 eluent at a flow rate of 0.6 mL/min. All of the sugar peaks were detected by the RI detector (Waters 2414, Waters Co., Milford, MA, USA).

3. Enzymatic Saccharification

Enzymatic saccharification was performed according to NREL Standard Procedures No.009 [24]. Commercial cellulases, Celluclast 1.5 L (Novozyme A/S Bagsvaerd, Denmark) and Novozym 188 (Novozyme A/S Bagsvaerd, Denmark), were obtained from Korea Institute of Energy Research (KIER, Korea), and had been purchased from Novozyme Korea Co., were used. Saccharification experiments were conducted in 150 mL Erlenmeyer flasks with a total working volume of 20 mL at a substrate concentration of 1% (w/v). The pre-warmed flask contained 50 mM sodium citrate buffer (pH=4.8), 80 μg/mL of tetracycline, and 60 μg/mL of cycloheximide to minimize microbial contamination. The commercial enzyme used in the study was a mixture of celluclast supplemented with Novozyme 188 β-glucosidase at a ratio of 1 : 2, FPU : CBU activities to alleviate end-product inhibition by cellobiose. The cellulase and β-glucosidase had activities of 80 FPU/mL and 480 CBU/mL, respectively. Reaction flasks were run in triplicate with the enzyme loadings of 60 FPU/g of glucan at 50 °C with 120 rpm rotation and compared to controls that contained 1% α-cellulose and untreated straw.

4. Simultaneous Saccharification and Fermentation (SSF)

Fermentation was performed in a manner similar to that described in NREL Standard Procedures [25]. The *Saccharomyces cerevisiae* DKIC was obtained from the Korea Culture Center of Microorganism (KCCM, Seoul, Korea). Yeast cells were grown in YP media [1% yeast extract (Becton-Dickinson and Company, Sparks, MD), 2% peptone (Becton-Dickinson and Company, Sparks, MD), 5% glucose (Sigma-Aldrich, Inc., St. Louis, MO)] at 30 °C for 16 h in a shaker incubator (Vision Scientific Co., Korea) at 200 rpm. In the SSF process, the fractionated straw was washed to remove the residual glucose and inhibitors. The slurry was diluted with deionized water to a final solid concentration of 2% (w/w). The diluted slurry was sterilized together with the fermentor in an autoclave at 121 °C for 20 min. Nutrients were sterilized, mixed together, and added to create final concentrations of 0.5, 0.025, and 1.0 g/L of $(NH_4)_2HPO_4$, $MgSO_4 \cdot 7H_2O$ and yeast extracts, respectively. The yeast cell suspension was added to a concentration of 1 g dry yeast cells/L. The SSF experiments were carried out in triplicate with the enzyme loadings of 8-45 FPU/g of glucan, using 1 L auto controlled fermentors (Biotron, Korea) with *Saccharomyces cerevisia* at a pH of 4.8 and at temperatures of 30, 34, and 37 °C. The pH was set using 0.05 M sodium citrate buffer before sterilization and also during SSF.

RESULTS AND DISCUSSION

1. Composition of Fractionated Barley Straw

Table 1 shows the analysis results of the barley straw based on the 105 °C dry weight (in our previous work). The initial composition

Table 1. Composition of the fractionated barley straw and mass balance in dilute-acid fractionation

Substrate	Solid remainig (%)	Solid (%)								Liquid (%)		EMB[a] (%)	
		Glucan	XMG	AIL[b]	Extractive		Crude protein	Ash	Total	Glucan	Xylan	Glucan	XMG
					EtOH	Hexan							
Untreated	100	37.8	21.5	18.9	9.2	1.8	2.8	2.6	94.7	-	-	-	-
Frationated	52.5	62.5	2.9	16.2	-	-	-	1.2	-			96.7	94.1
Frationated[c]		32.8	1.5	8.5	-	-	-	0.6	-	2.5	18.7	(−3.3)	(−5.9)
Component retention (%)		86.9	7.2	44.8	-	-	-	24.1	-	-	-	-	-

[a]Extraction mass balance (EMB)=$\{\Sigma C_{Li}-\Sigma C_{Si}\}/\Sigma C_{Ri}$; where C_i is the mass of each sugar component as C_{Li} determined through HPLC chromatography, the subscripts L, S and R refer to the extracted liquid, fractionated solids and raw straw fractions, respectively

[b]Acid insoluble lignin

[c]Analysis data are based on the oven dry untreated biomass

of the barley straw was determined to be: 37.8% glucan, 21.5% XMG (Xylan+Mannan+Galactan), and 2.5% arabinan. Carbohydrates accounted for 58.8% of the dry straw, which makes barley straw a very promising lignocellulosic biomass for ethanol production. The non-carbohydrate composition is 18.9% lignin, 9.2% ethanol (EtOH) extractive, 1.8% hexane extractive, 2.8% protein, 2.6% ash, and 1.1% other species. The composition of fractionated barley straw was determined to be: 62.5% glucan, 2.9% XMG, and 16.2% AIL (Acid Insoluble Lignin). Approximately 47.5% of the mass of the barley straw was solubilized into hydrolyzate. 18.7% XMG could be found in the hydrolyzate as xylose; in other words, XMG in the straw extracted into the hydrolyzate with a yield of 87.0%. The retention of glucan and XMG in fractionated barely straw was 86.9 and 7.2%, respectively, on the basis of untreated barley straw. The total extraction mass balance of sugar was 96.7% for glucan and 94.1% for XMG. Acid pretreatment of barley straw enabled a maximum solubilization of the hemicellulosic fraction with almost total XMG recovery. The pentose-rich hydrolyzate can be used for a five-carbon fermentation process and production of value added products, for example furfural, levulinic acid etc., with biorefinery procedures.

2. Enzymatic Saccharification

The saccharification of cellulose in fractionated biomass *via* enzymatic action is critical in releasing monomeric sugars for fermentation to bioethanol. The rate and extent of enzymatic saccharification of the fractionated straw provides a measure of the fractionation's effectiveness. As expected, the saccharification test resulted in a higher glucose yield than that of untreated barley straw. Fig. 1 shows the time course of the glucose yield for enzymatic saccharification of acid-fractionated barley straw. The glucose yield was expressed as g/g of glucan. In these experiments, the maximum saccharification of the fractionated barley straw was 70.8% after 72 h at 60 FPU/g of glucan, while the untreated sample was only 18.9% under the same conditions.

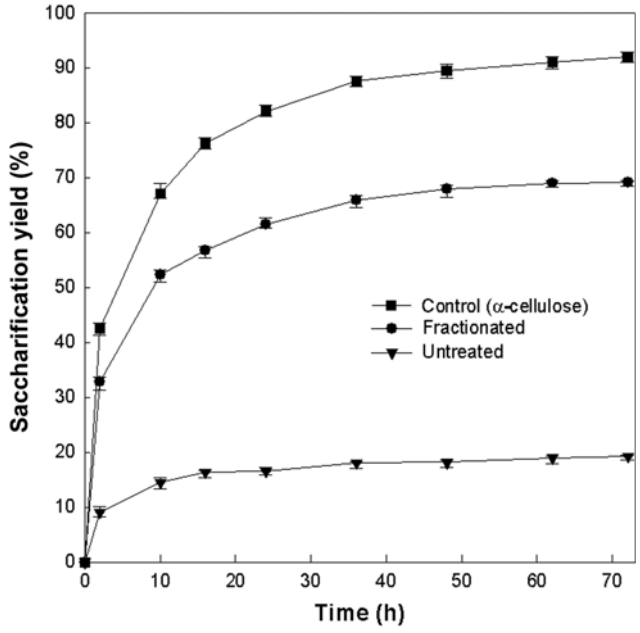

Fig. 1. Time course of the glucose yield for enzymatic saccharification of fractionated and untreated barley straw with α-cellulose as a control.

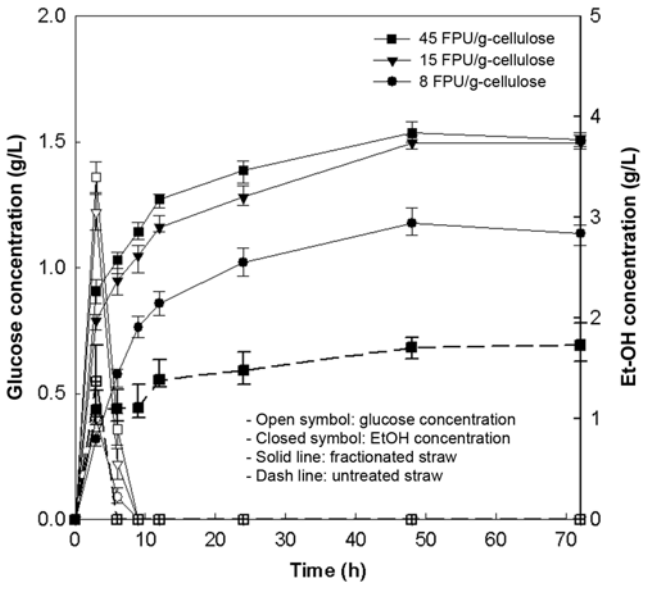

Fig. 2. Time courses of the glucose release and consumption, and ethanol production over 72 h simultaneous saccharification and fermentation (SSF) of fractionated barley straw with various enzyme loadings.

3. Optimization of SSF Process Variables

SSF was performed on the water-rinsed fractionated barley straw based on one gram of glucan with 100 mL of fermentation media using 1 g/L yeast at four different enzyme loadings; 8, 15, 30 (data not shown) and 45 FPU/g-glucan. Fig. 2 shows the concentration profiles of glucose and ethanol in SSF. On the assumption that perfect saccharification/fermentation have been performed, the theoretical ethanol yield is 35.06 g per 100 g fractionated straw, assuming that 1 g of glucose produces 0.51 g of ethanol. After 48 h SSF the ethanol concentration reached a plateau at 2.94, 3.74, and 3.84 g/L, at 8, 15, and 45 FPU/g-glucan enzymes loadings, and 1.66 g/L (45 FPU/g-glucan) for untreated straw. This corresponds to overall ethanol yields of 52.4%, 66.7%, 68.4%, and 29.6%, respectively. In SSF, at least after the first 10 h cultivation period, no further glucose accumulation was detected.

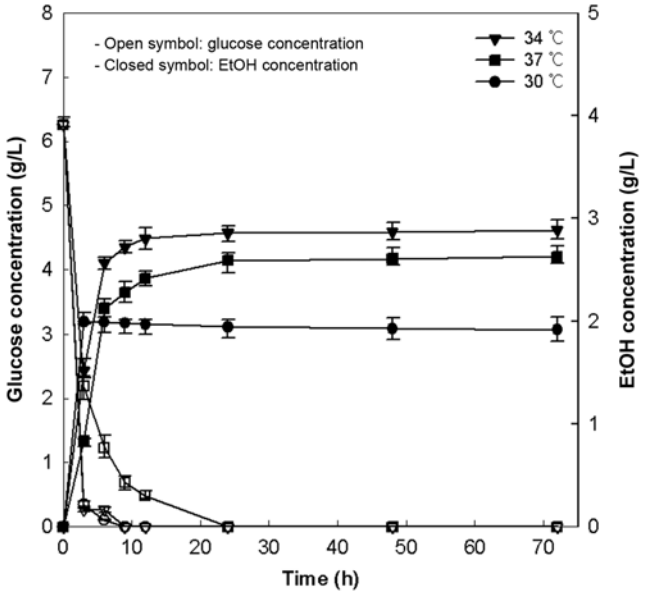

Fig. 3. Time courses of the glucose consumption and ethanol production over 72 h separate hydrolysis and fermentation (SHF) of fractionated barley straw at various reaction temperatures.

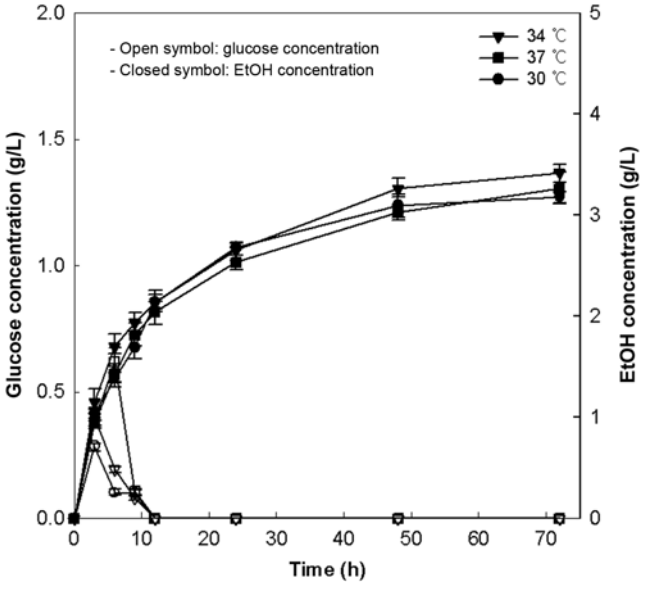

Fig. 4. Time courses of the glucose consumption and ethanol production over 72 h simultaneous saccharification and fermentation (SSF) of fractionated barley straw at various reaction temperatures.

Figs. 3 and 4 show the results of batch SHF and SSF experiments with different reaction temperatures at 30, 34, and 37 °C in order to investigate the influence of temperature on process performance. The impact of the temperature was much greater when SHF was carried out than for SSF. In the SSF process there was only a small difference in ethanol yield between the examined temperatures, but 37 °C was slightly less favorable than 30 and 34 °C. In the SHF, the initial glucose concentration in the mixture hydrolyzed by 15 FPU/g-glucan enzyme loadings started around 6.26 g/L, and the glucose levels decreased to almost zero very rapidly, in less than 24 h, with production of bioethanol. After 24 h SHF, the ethanol concentration reached a plateau at 2.64, 2.87, and 1.92 g/L, at 30, 34, and 37 °C. Whereas Fig. 4 shows that the influence of temperature was not much high in SSF, *i.e.*, all three temperatures gave similar ethanol yields, but with a slightly higher yield at 34 °C (3.18-3.42 g/L). However, it was shown to increase ethanol concentration slightly throughout the experiment. This indicates that the yeast cells were metabolically active during the entire course of the fermentation (72 h), as already observed by other authors [26-28].

4. Determination of the WIS Level

To obtain the desired WIS (water insoluble solid) level, the fractionated straw was folded in fermentation media with WIS levels of 2 to 10%. All experiments were carried out at 34 °C for 48 h with 15 FPU/g-glucan enzyme loading. Fig. 5 shows the highest glucose concentration of 29.14 g/L obtained from enzymatic saccharification with 6% WIS level of fractionated barley straw and this indicates a saccharification yield of 70.6%. At the same WIS level, a maximum ethanol concentration of 16.14 g/L, corresponding to a glucose

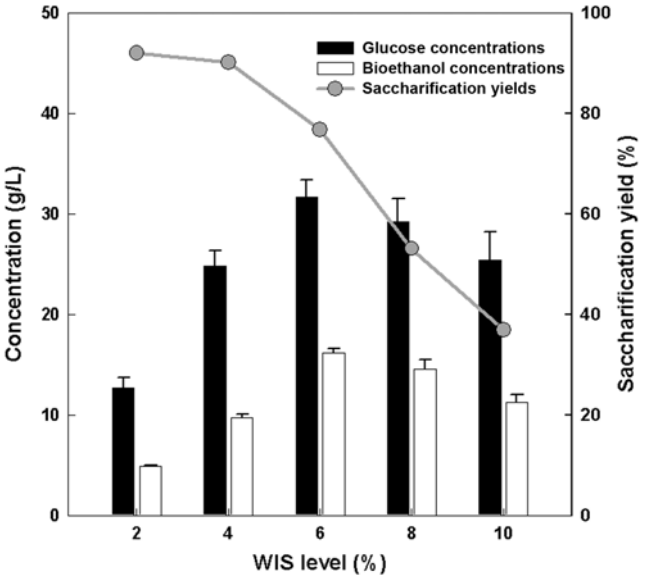

Fig. 5. Effect of water insoluble solid (WIS) contents on the performance of saccharification and ethanol production in SSF of fractionated barley straw.

equivalent concentration of 31.64 g/L, and an ethanol theoretical yield of 76.7% based on the total available glucose of fractionated barley straw were obtained from SSF. The difference of 2.5 g/L between the glucose equivalent concentration in SSF and the glucose concentration in SHF can be explained indirectly by noting that SSF is more effective than SHF for bioethanol production by more than at least 8.5%. In SHF and SSF at WIS levels greater than 6%, on the contrary, with increasing WIS level, the glucose concentration and ethanol production decreased because of inefficient mixing and enzyme deactivation by shear off.

5. Bioethanol Production through SSF

Fig. 6 presents the time profiles of the ethanol concentration during a batch SSF experiment at the aforementioned optimized conditions. After 72 h fermentation, the maximum ethanol concentration achieved from fractionated barley straw was 18.46 g/L, representing 87.8% of the maximum possible yield, while the untreated barley straw produced a yield of only 21.9% under the same conditions. In the case of SSF of untreated barely straw, bioethanol was produced rapidly until 12 h, but after that the production rate of bioethanol was almost zero. In the cellulose-rich fractionated barely straw, the fermented glucose equivalent was 36.2 g/L, whereas only 8.54 g/L of glucose equivalent was fermented with untreated barley straw. This means that enzymatic saccharification was the rate-limiting step for bioethanol production from cellulosic biomass for most of the duration of the SSF process.

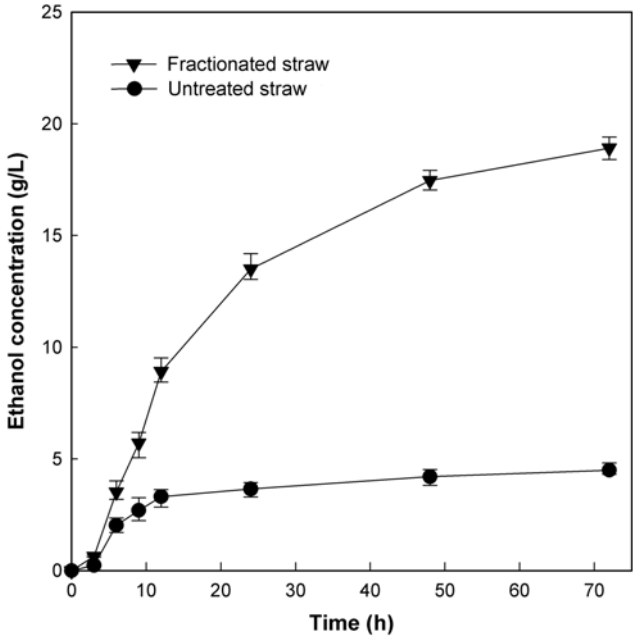

Fig. 6. Ethanol production by SSF with fractionated barley straw under the optimized conditions: 15 FPU/g of glucan enzyme loading, 6% of WIS content, and 34 °C.

6. Performance Comparison between SSF and SHF

Fig. 7 illustrates the overall mass balance based on the amount of glucose for each bioprocessing step. In this study, 6 g of fractionated barley straw, which has a glucan content of 62.5% (4.41 g glucose) was processed; it was obtained from 11.43 g of untreated barley straw through dilute acid-fractionation. With the enzymatic saccharification, 2.94 g of glucose was released and this corresponds to a saccharification yield of 70.5%. By following ethanol fermentation, 1.37 g of bioethanol was produced in the SHF process. In the SSF process, 1.63 g of bioethanol was produced, which corresponds to 3.47 g of glucose equivalent being fermented, and it corresponds to a saccharification yield of 83.2%. As a result, SSF was more effective for bioethanol production than SHF by around 18%.

CONCLUSION

Fractionation of barley straw with dilute acid produced a cellulose-rich and an easily digestible straw, which resulted in increases from 37.8 to 62.5% in glucan content and 18.9 to 70.8% in saccharification yield. The highest bioethanol concentration obtained in SSF was 18.46 g/L under optimized conditions: 6% of WIS level, 34 °C, and 15 FPU/g-glucan with yeast, *Saccharomyces cerevisiae* DKIC. The 18.46 g/L of ethanol concentration represented 92% of the theoretical ethanol yield and 83.2% of the saccharification yield, whereas the saccharification yield for SHF was 70.5%. For production of bioethanol, biomass pretreatment/fractionation and the

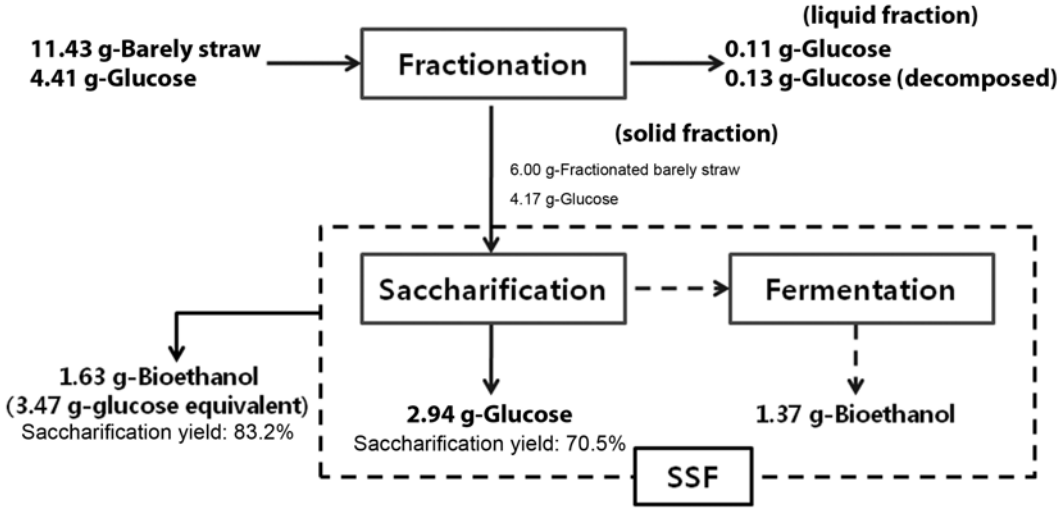

Fig. 7. Mass balance based on glucose amounts for bioethanol production by SHF and SSF.

cost of enzymes are critical targets for process and cost improvements. Therefore, this study predicts the effectiveness of the SSF process for barley straw with dilute acid-fractionation. However, this study revealed that the final ethanol concentration was not enough high; it needs to reach 4% (w/v) to be considered for reducing distillation costs [19,29]. Improving the efficiency of biomass fractionation for more cellulose-rich fractionated biomass, increasing the solid level in SSF process, and the development of non-isothermal SSF for creating environments where the enzyme is more active were impressed on.

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REFERENCES

1. M. Balat, *Energy Convers. Manage.*, **52**, 858 (2011).
2. Y. Matsumura, T. Minowa and H. Yamamoto, *Biomass Bioenergy*, **29**, 347 (2005).
3. FAOSTAT. Food and agriculture organization of the United Nations, http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor (Accessed Sep. 2011).
4. S. Kim and B. E. Dale, *Biomass Bioenergy*, **26**, 361 (2004).
5. Y. Chen, R. R. Sharma-Shivappa, D. Keshwani and C. Chen, *Appl. Biochem. Biotechnol.*, **142**, 276 (2007).
6. S. Brethauer, M. H. Studer, B. Yang and C. E. Wyman, *Bioresour. Technol.*, **102**, 6295 (2011).
7. M. Zeng, N. S. Mosier, C. P. Huang, D. M. Sherman and M. R. Ladisch, *Biotechnol. Bioeng.*, **97**, 265 (2007).
8. M. h. Han, Y. Kim, Y. Kim, B. Chung and G. W. Choi, *Korean J. Chem. Eng.*, **28**(1), 119 (2011).
9. L. Zhimin, Y. Liu, W. Liao, S. Chen and R. S. Zemetra, *Biomass Bioenergy*, **35**, 542 (2011).
10. C. Xiros, P. Katapodis and P. Christakopoulos, *Bioresour. Technol.*, **102**, 1688 (2011).
11. N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee and M. Holtzapple, *Bioresour. Technol.*, **96**, 673 (2005).
12. T. S. Jeong, B. H. Um, J. S. Kim and K. K. Oh, *Appl. Biochem. Biotechnol.*, **161**, 22(2010).
13. K. Olofsson, M. Bertilsson and G. Liden, *Biotechnol. Biofuels*, **1**, 1 (2008).
14. X. Lu, Y. Zhang, Y. Liang, J. Yang, S. Zhang and E. Suzuki, *Korean J. Chem. Eng.*, **25**(2), 302 (2008).
15. C. E. Wyman, D. D. Spindler and K. Grohmann, *Biomass Bioenergy*, **3**(5), 301 (1992).
16. L. Peng and Y. Chen, *Biomass Bioenergy*, **35**, 1600 (2011).
17. J. Söderström, M. Galbe and G. Zacchi, *J. Wood Chem. Technol.*, **25**(3), 187 (2005).
18. K. Öhgren, M. Galbe and G. Zacchi, *Process Biochem.*, **42**(5), 834 (2006).
19. A. Wingren, M. Galbe and G. Zacchi, *Biotechnol. Prog.*, **19**(4), 1109 (2003).
20. A. Wingren, M. Galbe and G. Zacchi, *Bioresour. Technol.*, **99**(7), 2121 (2008).
21. T. Ehrman, Chemical Analysis & Testing Standard Procedure., No. 002 (1992).
22. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Tmpleton and D. Crocker, NREL/TP-510-42618 (2010).
23. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Tmpleton, NREL/TP-510-42623 (2008).
24. A. Sluiter, D. Hyman, C. Payne and J. Wolfe, NREL/TP-510-42627 (2008).
25. M. Selig, N. Weiss and Y. Ji, NREL/TP-510-42629 (2008).
26. N. Dowe and J. Mcmillan, NREL/TP-510-42630 (2008).
27. B. H. Um and S. H. Bae, *Korean J. Chem. Eng.*, **28**(5), 1172 (2011).
28. H. Ooshima, Y. Ishitani and Y. Harano, *Biotechnol. Bioeng.*, **27**, 389 (1985).
29. G. P. Philippidis and T. K. Smith, *Appl. Biochem. Biotechnol.*, **51**, 117 (1995).
30. K. K. Oh, S. W. Kim, Y. S. Jeong and S. I. Hong, *Appl. Biochem. Biotechnol.*, **89**, 15 (2000).
31. M. Ballesteros, J. M. Oliva, M. J. Negro, P. Manzanares and I. Ballesteros, *Process Biochem.*, **39**, 1843 (2004).