Bioethanol Production from *Ulva pertusa* Kjellman by High-temperature Liquefaction

W. Y. Choi,[a] J. G. Han,[a] C. G. Lee,[a] C. H. Song,[a] J. S. Kim,[b] Y. C. Seo,[b] S. E. Lee,[c] K. H. Jung,[c] D. H. Kang,[d] S. J. Heo,[d] J. S. Cho,[e] and H. Y. Lee[a,*]

[a]College of Bioscience & Biotechnology, Kangwon National University, Chucheon 200-701, Korea

[b]Medical & Bio-Material Research Center and Department of Biomaterials Engineering, Kangwon National University, Chuncheon 200-701, Korea

[c]Department of Biotechnology, Chungju National University, Jeungpyung, Chungbuk, 368-701, Korea

[d]Korea Ocean Research & Development Institute, Ansan P.O. Box 29, Seoul 426-744, Korea

[e]DooSan EcoBizNet, Chucheon 200-161, Korea

Original scientific paper
Received: July 28, 2011
Accepted: February 27, 2012

This work was investigated to improve hydrolysis yields of macro alga, *Ulva pertusa* Kjellman by high-temperature liquefaction process (HTLP). We hydrolyzed this alga to produce bioethanol. *U. pertusa* Kjellman contains approximately w = 32 % glucose, comprising w = 6 % cellulose and 20 % starch, along with w = 5.9 % xylose. Among 32 % of total carbohydrates, ca. 26 % of glucose was hydrolyzed from starch (20 %) and cellulose (6 %), respectively, which tells that a more efficient process might be considered to completely hydrolyze the polymers containing fermentable sugars such as glucose and galctose, etc. Optimal hydrolysis conditions for the high-temperature liquefaction process (HTLP) were determined to be 15 MPa and 150 °C for 15 min, with water as the solvent. We found that the process temperature and time were the most important factors in the operation. Under these conditions, the conversion yields of glucose and xylose were 9.08 and 21.14 %, respectively. After cellulase and amyloglucosidase treatment, 61.1 % glucose (based on w = 32.1 %, dry basis) was converted into glucose without further conversion into xylose. The present process provided 3.1 to 12.6 % higher overall hydrolysis yields from *U. pertusa* Kjellman than those from other agricultural biomass. The HTLP process generated only about 40 mg L^{-1} of HMF (5-hydroxymethylfurfural). This concentration was much less than those from other pretreatment processes and resulted in approximately 90 % of the maximum theoretical ethanol yield. In addition, the hydrolysis pattern of *U. pertusa* Kjellman was much different from those of agricultural biomass materials due to different starch compositions and polymer structures.

Key words:
Bioethanol, *U. pertusa* Kjellman, High-Temperature Liquefaction Process (HTLP), high converted yield, different sugar compositions

Introduction

The recent instability and high prices of global petroleum supplies have aggressively driven the development of alternative energy sources such as cellulosic biomass, which is composed of mainly cellulose, hemicellulose and lignin.[1] However, the widespread production of ethanol from cellulosic biomass is hindered by several fundamental issues, including deforestation, loss of biodiversity, low energy output/input-balance and social issues like human displacement.[2,3] To overcome these problems, marine biomass has been pursued as a competitive bioenergy resource because the oceans are home to 80 % of Earth's flora and fauna, in which 90 % of global photosynthesis occurs. Furthermore, marine biomass does not compete with agricultural food and feed production.[4,5] Algae have the advantage of having no lignin and low hemicellulose levels, which results in an increased hydrolysis efficiency and/or fermentation yields.[6] The use of marine biomass for bioenergy could also reduce environmental problems in the sea because some sea pollutants could be utilized as bioethanol biomass, such as *Ulva pertusa* Kjellman.[7,8] The green macroalga *U. pertusa* Kjellman is a major sea pollutant in the far-east and southeast areas. The alga contains approximately w = 47.0 % total carbohydrates, in the form of several types of polysaccharides, and low levels of cellulose, which is present only in the cell wall.[6,9,10] Consequently, the hydrolysis process for marine biomass should be different from conventional pretreatment techniques for the saccharification of cellulosic materials. There are various

*Corresponding author: Tel: +82-33-250-6455; Fax: +82-33-250-6560; E-mail: HYEONL@kangwon.ac.kr

ways to hydrolyze biomass depending on the type and composition.[11] The most widely used hydrolysis processes for agricultural cellulosic biomass are 1) thermochemical treatment using dilute acid with or without rapid steam decompression and 2) ammonia pretreatment, which is generally followed by cellulase treatment.[11,13] Because acid and/or alkali treatment with moderate thermal processes at 100 – 120 °C digest only hemicelluloses, the cellulose must be further hydrolyzed by cellulase treatment to efficiently obtain fermentable sugar, i.e., glucose.[12,13]

However, it has been shown that these processes cannot effectively hydrolyze several kinds of algae due to their different cell wall structures and/or more complex types of sulfated polysaccharides.[6,10,14] Instead, high-temperature liquefaction (HTP) under high pressure has been employed to achieve the relatively efficient hydrolysis of algae into glucose for bioethanol production. The denaturation of fiber proteins occurs under high-temperature and high-pressure conditions, leading to the destruction of cell membranes and an increased porosity that allows solvents to freely access the cell contents. This process ultimately improves the hydrolysis efficiency of polysaccharides, mainly with respect to amylopectin structure.[6,10,11,14,15] Additionally, this process might have the advantage of generating less hydroxymethylfurfural (HMF) and other fufurals degraded from xylose due to the acid and harsh pretreatment conditions; the reduced amounts of toxic residues in the hydrolysates could help increase alcohol fermentation yields.[16,17] Therefore, in this work, the HTP process was optimized to hydrolyze *U. pertusa* Kjellman for bioethanol production, and the results are compared to those produced by utilizing agricultural cellulosic biomass.

Material and methods

Materials

Ulva pertusa Kjellman was harvested from June to August 2008 on Jeju Island, Korea, and 20 kg of *Ulva pertusa* Kjellman was dried in a chamber air dryer (OF-22GW, Korea) at 40 °C for 48 hours. It was then ground into a powder with a ball mill, and stored in a freezer at –20 °C before use.

Monosaccharide composition analysis of *U. pertusa* Kjellman

To analyze the monosaccharide content in *U. pertusa* Kjellman, 10 g of dried *U. pertusa* Kjellman was treated with 1 eq/L NaOH at 100 °C for 3 – 4 hours, and then 1 eq/L H_2SO_4 was added at 120 °C. The hydrolyzed solution was treated with 1 eq/L trifluoroacetic acid (TFA) solution at 120 °C for 2 hours and then neutralized with 2 eq/L NaOH for HPLC analysis. HPLC (Waters 510, Waters, MA, USA) with an RI-detector (Waters 410, Waters, MA, USA) and carbohydrate column (4.6 × 250 mm, Waters, MA, USA) was performed with 80 % acetonitrile as the eluent at 2 mL min^{-1}.[18] Solutions of glucose, xylose, mannose, fructose and galactose (all 99 % purity; Sigma, St. Louis, MO, USA) were prepared as standards.

For cellulose and hemicellulose analysis, 10 g of *U. pertusa* Kjellman powder was reacted with 0.05 mol L^{-1} acetic acid and 1.3 % $NaClO_2$ solution at 75 °C for 1 hour. Following centrifugation at 10,000 rpm for 10 min, the supernatant was removed. The pellet was oven-dried at 110 °C for 48 hours to obtain holocellulose.[18] Next, 500 mg of the holocellulose was incubated with 4 mol L^{-1} NaOH for 2 hours. The solution was centrifuged at 10,000 rpm for 10 minutes, and the supernatant removed. The pellet was washed with distilled water and dried again at 110 °C for 48 hours to obtain cellulose. The amount of hemicellulose was calculated by subtracting the amount of cellulose from the amount of holocellulose.[19]

Pretreatment of *U. pertusa* Kjellman by high-temperature liquefaction

U. pertusa Kjellman powder was hydrolyzed with hot water in a pressurized vessel for the high-temperature liquefaction process (HTLP). Fig. 1 shows a schematic diagram of the batch-type HTLP reactor, which consisted of a 1–L reaction vessel, a nitrogen gas injection booster, a temperature gauge (TG), a pressure gauge (PG) and three safety check valves. About φ = 10 % *U. pertusa* Kjellman was loaded in the vessel and heated to 400 °C and 40 MPa. N_2 gas was used for pressure transmission. The reaction was put under a vacuum and backfilled with nitrogen to eliminate interfer-

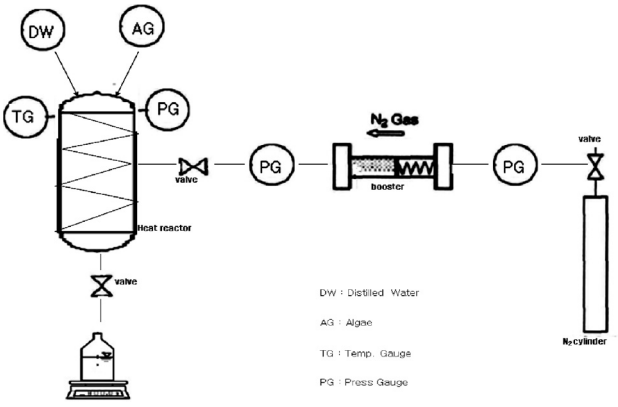


Fig. 1 – *Schematic of the batch-type high-temperature liquefaction system*

ence from the air and to increase the hydrolysis efficiency. The operating ranges of the three key parameters of temperature, process time and pressure were designated as 90, 120 or 150 °C at 5 to 30 MPa for 5 – 15 min. Generally, inprevious experiments most monosaccharides would be broken down under supercritical and near-supercritical conditions.

Enzyme treatment of the pretreated residues

The reaction solids were further hydrolyzed with cellulase after separating the liquid from the HTLP by centrifugation at 1,500 rpm. The residues were reacted with 30 FPU g^{-1} cellulase (Cellubrix L, 96 IU FPA mL^{-1}; Novozyme, A/S, USA) and amyloglucosidase (400 units mL^{-1}) in 100 mL of sodium acetate buffer (pH 4.8) at 50 °C and 150 rpm for 24 hours. The reaction was then analyzed by HPLC (Waters, USA) to estimate the glucose and xylose concentrations.[18]

Measurement of glucose, xylose and 5-hydroxymethylfurfural (HMF) concentrations in the hydrolysates

For the quantitative analysis of glucose and xylose in the hydrolyzed solutions from the HTLP and enzymatic treatment processes, the same conditions were applied as in the analysis of monosaccharides (see above): HPLC (Waters 510, Waters, USA) with an RI-detector (Waters 410) and a carbohydrate analysis column (4.6 × 250 mm, Waters, USA) eluted at 2 mL min^{-1} with 80 % acetonitrile as the mobile phase.[19] 5-Hydroxymethylfurfural (HMF), an inevitable major toxic residue from the degradation of xylose during high-temperature and/or acidification processes, was also quantified by HPLC as follows: an Aminex HPX-87H column (Bio-Rad, USA) and RI detector were used with 0.5 mL min^{-1} of 5 mmol L^{-1} H_2SO_4 as the mobile phase at 65 °C.[16,17,19,20]

Ethanol production from the pretreated hydrolysates

To confirm ethanol production from the hydrolysates, 100-mL aliquots of the liquid from both processes were fermented by *Saccharomyces cerevisiae* (ATCC 24858) in modified YPD medium (1 % yeast extract and 2 % peptone, pH 5.5) in a 500-mL flask at 30 °C and 150 rpm for 24 hours. Ethanol concentrations in the culture broth were measured by gas chromatography (HP-5890II, Agilent, USA) with a flame ionization detector (FID) at a 150 °C oven temperature and 250 °C injector and FID temperatures. N_2 gas was used as the carrier gas at a flow rate of 50 mL min^{-1}, and an INNO-Wax column (30 m × 0.32 mm, Agilent, USA) was used. *n*-Butanol was used as an internal standard.[21]

Results and discussion

Monosaccharide composition of *U. pertusa* Kjellman

Table 1 shows the total sugar and monosaccharide compositions of *U. pertusa* Kjellman. The total sugar content was estimated to be w = 38.4 %, and the mass fractions of glucose and xylose were 83.5 and 15.4 %, respectively, based on the total sugar content. Total sugar was analyzed since *U. pertusa* Kjellman consists of various carbohydrates such as cellulose, hemicellulose, starch and other polysaccharides.[26,27] Thus, the starch content of *U. pertusa* Kjellman is considerable, suggesting that this alga could be an excellent source of biomass for bioethanol production if it is properly hydrolyzed. According to these sugar profiles, w = 32.1 % of the maximum theoretical glucose conversion yield could be obtained by hydrolyzing *U. pertusa* Kjellman. This yield was similar to the glucose conversion from corn stover (36.1 %) and much higher than that from *Sargassum sagaminanum* (19.7 %), whose marine biomass are most widely investigated to produce bioethanol.[31,32] However, most of the glucose was in the form of polysaccharides: the cellulose, hemicellulose and starch contents were only w = 6.7 %, w = 16.8 % and w = 20.1 %, respectively (not shown in Table 1). Thus, the conventional pretreatments used for agricultural biomass cannot be employed to hydrolyze *U. pertusa* Kjellman. Alternative pretreatment processes should be developed to more effectively hydrolyze the complex polysaccharide structures that do not dissolve well in hot water, dilute acids and alkaline environments. Interestingly, *U. pertusa* Kjellman does not contain large amounts of lignin, which resulted in the production of 5-hydroxymethylfurfural (HMF) during the pretreatment process, as shown in Table 5. These data indicate that this high pressure liquefaction process is very efficient in generating less amounts of HMF even though only very small amounts of ligin exist in *U. pertusa* Kjellman.[28–30]

Table 1 – *Analysis of total fermentable sugar compositions of U. pertusa (w/%)*

Total carbohydrates	Monosaccharide composition†				
	fuc	xly	man	glu	gal
38.4±0.14*	–	15.4±0.79	1.1±0.25	83.5±0.41	0.1±0.07

†Monosaccharide compositions were expressed as mass fractions based on the total carbohydrates in *U. pertusa*.

*Each value is presented as the mean ± S.E. of three independent experiments.

Hydrolysis yields after high-temperature liquefaction process pretreatment and enzymatic treatment

Tables 2, 3 and Fig. 2 show the glucose and xylose conversion yields after HTLP pretreatment and after treatment with cellulase (30 FPU g^{-1}) and amyloglucosidase (400 units mL^{-1}) to compare the hydrolysis efficiencies under various pretreatment conditions. Based on the data shown in Table 2, the process temperature and time, but not the pressure, had the most impact on the glucose and xylose concentrations in the hydrolysates. The optimized treatment conditions were determined to be 150 °C and 15 MPa for 15 min. Under the optimized condi-

Table 2 – *Estimation of glucose and xylose hydrolysis conversion yields in the hydrolysates through the high temperature liquefaction process*

Temp. (°C)	Time (min)	Pressure (MPa)	Glucose* (*w*/%)	Xylose* (*w*/%)
90	5	5	7.49±0.11†	17.27±0.95†
90	5	15	7.61±0.21	17.10±0.87
90	5	30	7.61±0.19	17.94±0.79
120	10	15	7.90±0.30	19.15±0.29
120	10	30	7.90±0.35	19.99±0.39
120	10	5	8.39±0.34	20.70±0.45
150	15	30	7.80±0.11	20.29±0.21
150	15	5	8.30±0.29	20.87±0.10
150	15	15	9.08±0.19	21.14±0.09

*Each value is presented as the mean ± S.E. of three independent experiments.

†A single value indicates release monomers. Hydrolysis conversion yields were calculated based on the sugars in *U. pertusa* with the maximum glucose and xylose concentrations as w = 32.06 % and w = 5.91 %, respectively.

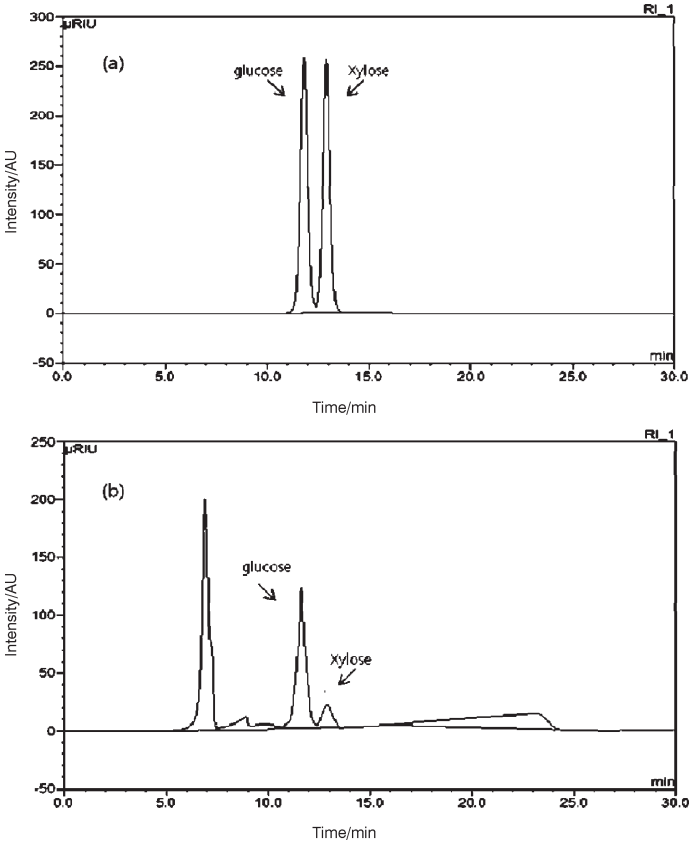


Fig. 2 – *HPLC chromatogram of standard and hydrolysate: (a) standard (b) hydrolysate*

Table 3 – *Estimation of glucose and xylose hydrolysis conversion yield in the residues after cellulase and amyloglucosidase treatment*

Temp. (°C)	Time (min)	Pressure (MPa)	Cellulase†		Amyloglucosidase††	
			glucose (*w*/%)	xylose (*w*/%)	glucose (*w*/%)	xylose (*w*/%)
90	5	5	78.11±0.11*	0.76±0.23	30.87±0.65	0.13±0.25
90	5	15	79.98±0.81	0.84±0.71	31.66±0.39	0.15±0.14
90	5	30	84.66±0.34	0.29±0.84	35.64±0.51	0.14±0.09
120	10	15	87.16±0.45	0.37±0.17	36.53±0.37	0.11±0.22
120	10	30	88.40±0.16	0.53±0.32	38.59±0.15	0.20±0.13
120	10	5	88.09±0.83	0.07±0.64	38.34±0.42	0.22±0.19
150	15	30	88.71±0.87	0.29±0.53	38.43±0.54	0.21±0.15
150	15	5	89.34±0.68	0.91±0.28	39.14±0.19	0.24±0.23
150	15	15	98.59±0.44	0.91±0.14	40.55±0.21	0.26±0.11

*Each value is presented as the mean ± S.E. of three independent experiments.

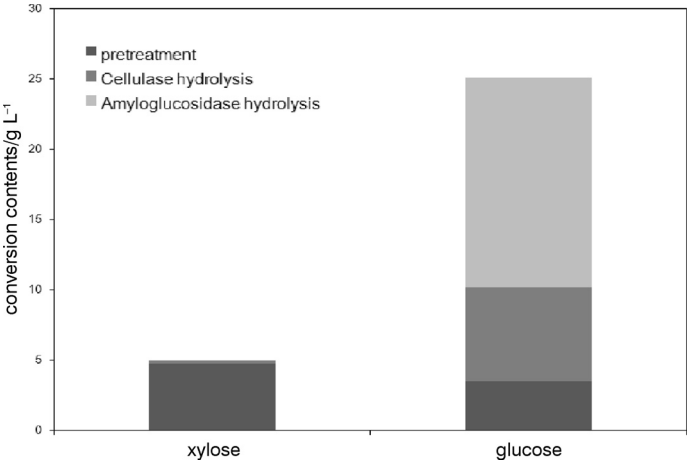
†A single value indicates release monomers. Conversion yields were calculated based on w = 6.7 % and w = 16.8 % of maximum cellulose and hemi-cellulose contents in *U. pertusa.*

††A single value indicates release monomers. Conversion yields were calculated based on w = 20.01 % of maximum starch contents in carbohydrate.

tions, the polymers in *U. pertusa* Kjellman seemed to be more easily hydrolyzed into xylose than glucose, as shown by the 9.08 % glucose and 21.14 % xylose conversion yields based on theoretical maximum concentrations from the input dry biomass. The hydrolysis yield of glucose derived from complex polysaccharides was low, but most of the xylose was hydrolyzed. This result indicates that the conditions used in our study are optimal for the hydrolysis of xylose from complex polysaccharides in *U. pertusa* Kjellman. The high xylose conversion yields observed in this study may be due to the relatively small amounts of xylose and hemicellulose in *U. pertusa* Kjellman compared with that of agricultural biomass. However, a w = 9.08 % glucose conversion yield from *U. pertusa* Kjellman has never before been reported with only pressurized hot water treatment; in fact, this yield was even higher than those reported for corn stover (3 – 7 %) with only pretreatment processes.[22,23]

In general, most of the glucose in hydrolysates should be destroyed at temperatures above 150 °C under acidic conditions; thus, the 9.08 % glucose conversion yield in our study implies that there are certain limitations to increasing the process temperature, especially for marine biomass, which, unlike cellulosic biomass, contains many types of sugars. Most common pretreatment processes are acid treatments followed by several kinds of enzymatic treatment, with hydrolysis yields of 30 % on average. That indicates that HTLP should be applied to increase the conversion yields by considering the characteristics of the polymers in macro alga, not using chemicals. The results shown in Table 3 demonstrate the effectiveness of HTLP: the cellulose and carbohydrates in the hydrolysates were completely hydrolyzed into glucose and not xylose by treatment with cellulase and amyloglucosidase. Furthermore, these results are superior to those for corn stover and other cellulosic biomass. As compared with low-temperature treatments, cellulose seemed to be more easily hydrolyzed upon enzyme treatment at about 150 °C, showing 99 % conversion yields from a total w = 9.08 % cellulose in *U. pertusa* Kjellman. These results indicate that HTLP can loosen and completely hydrolyze complex *U. pertusa* Kjellman cellulose and polysaccharides to allow efficient enzymatic conversion. This tendency was similar to the pretreatment cases shown in Table 2. These results indicate that HTLP would be an appropriate process for hydrolyzing marine biomass, despite the low conversion yield.

Fig. 3 shows a quantitative comparison of the total amounts of glucose and xylose acquired from both processes based on a total input of 100 g of dry biomass in a 1 L reactor. As shown in Tables 2 and 3, a high conversion yield of xylose (4.8 g L^{-1}) was obtained after HTLP pretreatment, with no xylose being generated after enzymatic treatment, while about 3.52 and 21.53 g L^{-1} of glucose was obtained from the HTLP and enzymatic treatments, respectively. In total, we obtained about 21.53 g L^{-1} of glucose from these processes, which was somewhat low compared to the 20–30 % total conversion yields from agricultural cellulosic biomass subjected to acid or alkali pretreatments followed by cellulose treatment.[16] In comparing the glucose and xylose hydrolysis yields of HTLP, it was evident that the yield is dependent on the reaction temperature and time, yet independent of pressure. However, considering that HTLP uses no kind of acid or other chemicals, and is a relatively simple process, a 48.84 % total sugar conversion yield is impressive, even though this process should be further optimized to increase the conversion yields.



F i g . 3 – *Total sugar concentrations in U. pertusa Kjellman hydrolysates after high-temperature liquefaction pretreatment and enzymatic hydrolysis. The total hydrolysate mass fraction yields were calculated based on the sugar released from U. pertusa Kjellman, with a maximum potential mass fraction yield of glucose and xylose of w = 32.06 % and 5.91 %, respectively.*

Shown in Table 4 are the hydrolysis yields of HTLP for *U. pertusa* Kjellman compared with various hydrolysis processes for agricultural biomass (results for other types of marine biomass have not yet been reported). Specifically, the results of this study were compared with the results of processes using water as a solvent, such as DCF (flow-through and compressed hot-water pretreatment) of corn stalks, because HTLP also uses only water. [19,24,25] This comparison shows the difficulty in hydrolyzing lignin in short periods of time using only water. However, with acidic or alkali pretreatments, the enzymatic reaction is inhibited by the byproducts of the pretreatments, leading to poor efficiency and low economic feasibility.[24,25] Furthermore, there are other problems, such as equipment corrosion and the wastewater treatment required for the high-temperature process.[11,15] We were surprised

Table 4 – *Comparison of hydrolysate sugar yields after pretreatment and enzymatic hydrolysis.*

Methods	Pretreatment		Enzymatic hydrolysis		Total conversion yield	
	xylose (%)	glucose (%)	xylose (%)	glucose (%)	xylose (%)	glucose (%)
High-temp. liquefaction†	21.1	9.1	0.9	61.1	21.9	64.6*
DCF††	31.5	6.7	1.2	52.2	32.7	57.9
Flow Through‡ (Liu and Wyman, 2005)	1.7	4.5	0.7	57.0	2.4	61.5
Compressed-hot water‡ (Liu and Wyman, 2005)	0.9	1.5	6.3	50.5	7.2	52.0

†High temp. liquefaction process were operated at 150 °C and 50 MPa for 15 min with water and 30 FPU g^{-1} glucan celluase, 2 mL amyloglucosidase (400 units mL^{-1}) was loaded.

††A loading of 60 FPU g^{-1} glucan for DCF, flow-through and compressed-hot water pretreatment method (Liu and Wyman, 2005).

‡DCF, flow-through and compressed-hot water reported in each column was released into solution. Yields are defined based on the maximum potential sugars released from corn stover of w = 59.7 % of dry solids with the maximum potential xylose being w = 37.7 % and the maximum potential yield of glucose being w = 62.3 % on this basis. A value indicates release of only monomers (Liu and Wyman, 2005).

to find that HTLP pretreatment produced an estimated glucose yield of about 9.1 %, which is much higher than that from other processes.

In relation to the enzyme treatment, the HTLP process hydrolyzed 98 % of the cellulose in the biomass, despite the cellulase being used at a relatively low specific activity of 30 FPU g^{-1}, as opposed to the 60 FPU g^{-1} used in other conventional processes. This yield is much higher than that from other processes listed in Table 4, and is likely due to the lower levels of cellulose and hemicellulose in *U. pertusa* Kjellman. Lower levels of these polysaccharides would reduce the crystallization of fiber proteins, leading to an increase in enzyme absorption during the high-pressure liquefaction and cellulase treatments.

Analysis of 5-HMF and ethanol production of hydrolysates

Because many phenolic compounds are produced during cellulose and lignin hydrolysis, it is important to minimize the amounts of toxic compounds, including 5-HMF, produced during alcohol fermentation pretreatment. As shown in Table 5, HTLP generally produced only small amounts of toxic residues. As previously highlighted, because *U. pertusa* Kjellman does not contain lignin and possesses low levels of cellulose and hemicellulose, lower HMF formation was expected during the hydrolysis process. The low production of HMF may also have been due to the gentle increase in reaction temperature by the batch-type HTLP reactor.[11,15] Under our optimized conditions for hydrolyzing biomass into glucose (50 MPa and 150 °C for 15 minutes), the lowest amount of 5-HMF was obtained: 36.6 ± 0.37 mg L^{-1}, which is a very low concentration. We found that the treatment pressure did not significantly affect HMF generation, as shown in Table 2 and reported previously.[22] To determine whether the hydrolysates contained any other toxic residues after fermentation, we measured the alcohol production from both the HTLP-pretreated and enzymatically treated hydrolysates (Fig. 4). As shown in Tables 2 and 3, a total of 25.05 g L^{-1} of

Table 5 – *HMF concentrations in the hydrolysates after high temperature liquefaction process according to pretreatment conditions*

Temp. (°C)	Time (min)	Pressure (MPa)	HMF (mg L^{-1})*
90	5	5	40.1±0.91
90	5	15	35.7±0.44
90	5	30	42.6±0.76
120	10	15	39.1±0.46
120	10	30	33.5±0.74
120	10	5	38.6±0.41
150	15	30	29.2±0.45
150	15	5	31.9±0.15
150	15	15	36.6±0.37

*Each value is presented as the mean ± S.E. of three independent experiments.

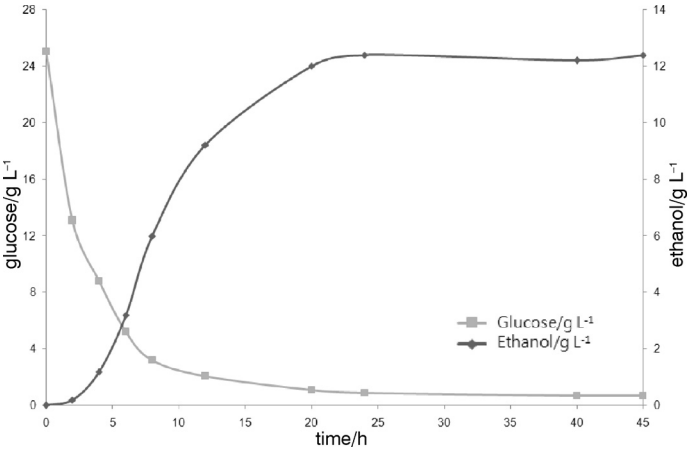


Fig. 4 – *Kinetics of glucose consumption and ethanol production during hydrolysate fermentation*

glucose was obtained through the two processes; this glucose was converted into about 12.4 g L^{-1} of ethanol during the 40-hour fermentation with a short lag phase, suggesting that the hydrolysates yielded approximately 90 % of the theoretical maximum ethanol production from glucose.

Conclusion

These features, combined with the increase in cellulose porosity and reactivity with cellulase caused by HTLP, ultimately promote the effective hydrolysis of complex polysaccharides in *U. pertusa* Kjellman. With respect to the total glucose hydrolysis yield, HTLP can more efficiently hydrolyze marine biomass than other processes using water, even though the overall glucose yield was somewhat lower than that from processes using agricultural biomass. In particular, most of the glucose in the hydrolysates could be used to produce ethanol, whereas the HTLP generated no toxic inhibitory residues during alcohol production, most probably due to the non-lignocellulosic structures of *U. pertusa* Kjellman. In other words, all of the glucose contained in the hydrolysates can be converted into ethanol when HTLP efficiently hydrolyzes the polysaccharides in *U. pertusa* Kjellman into glucose. Future studies should include a detailed comparison of these processes using the same type of marine biomass. Regardless, it is clear that HTLP is an efficient and promising process for treating marine biomass. Unlike terrestrial sources of biomass, the oceans provide an almost unlimited supply of biomass that can be utilized as an economically viable source of bioenergy. However, more systematic research needs to be conducted to optimize the reaction temperature, pressure and time in order to develop a feasible and efficient method of bioethanol production from marine biomass.

ACKNOWLEDGEMENTS

Authors deeply appreciate their financial support from Korea Institute of Energy and Resources Technology Evaluation and Planning.(PE98592)

Nomenclature

w – mass fraction, %

φ – volume fraction, %

References

1. *Wright, L.*, Biomass Bioenergy **30** (2006) 706.
2. *Hill, J., Nelson, E., Tilman, D., Polasky, S., Tiffany, D.,* Proc. Natl. Acad. Sci. U.S.A. **103** (2006) 11206.
3. *Goldemberg, J.,* Science **315** (2007) 808.
4. *Ray, B., Lahye, M.*, Carbohydr. Res. **274** (1995) 251.
5. *Demirbas, A.,* Prog. Energy Combust. Sci. **33** (2007) 1.
6. *Kloareg, B., Quatrano, R. S.,* Oceanography. Marine. Biol. **26** (1988) 259.
7. *Steinmetz, K. A., Potter, J. D*., Cancer Causes Control **2** (1991) 427.
8. *Floreto, E. A. T., Hirata, H., Ando, S., Yamasaki, S.,* Botanica Marina. **36** (1993) 217.
9. *Yu, P., Zhang, P., Li, N., Xu, Z., Wang, Y., Li, Z*., J. Appl. Phycol. **15** (2003) 21.
10. Percival, E., Br. Phycol. J. **14** (1979) 103.
11. *Zhang, S., Zhu, J., Wang, C*., Int. J. Pharm. **278** (2004) 471.
12. *Zhu, J. Y., Pan, X. J., Wang, G. S., Gleisner, R*., Bioresour. Technol. **100** (2009) 2411.
13. *Palmqvist, E., Hahn-Hägerdal, B*., Bioresour. Technol. **74** (2000) 17.
14. *Newmark, H. L*., Bull. Kor. Soc. Fish. Technol. **19** (1996) 502.
15. *Bennett, P. B., Marquis, R. E., Demchenko, I.,* University of Rochester Press **1** (1998) 482.
16. *Palmqvist, E., Grage, H., Meinander, N. Q., Hahn-Hägerdal, B*., Biotechnol. Bioeng. **63** (1999) 46.
17. *Klinke, H. B., Thomsen, A. B., Ahring, B. K.,* Appl. Microbiol. Biotechnol. **66** (2004) 10.
18. *Jingjing, Z., Quanbin, Z., Jing, W., Xuelian, S., Zhongshan, Z.,* Chin. J. Oceanol. Limnol. **27** (2009) 578.
19. *Lishi, Y., Hongnam, Z., Jingwen, C., Zengxinag, L., Qiang, J., Honghua, J., He, H.,* Bioresour. Technol. **100** (2009) 1803.
20. *Larsson, S., Palmqvist, E., Hann-Hagerdal, B., Tengborg, C., Stenberg, K., Zacchi, G.,* Enzyme Microb. Technol. **24** (1999) 151.
21. *Seo, H. B., Kim, S. S., Lee, H. Y., Jung, K. H.,* Mycobiol. **37** (2009) 133.
22. *Chaogang, L., Wyman, C. E.,* Bioresour. Technol. **96** (2005) 1978.
23. *Sun, J., Xu, X., Sun, F., Xiao, X. B., Sun, R. C*., Polym. Degrad. Stab. **88** (2005) 521.
24. *Liu, C., Wyman, C. E.,* Ind. Eng. Chem. Res. **42** (2005) 5409.
25. *Liu, C., Wyman, C. E.*, Bioresour. Technol. **96** (2005) 1978.
26. *Park, J. I., Woo, H. C., Lee, J. H.,* Korean Chem. Eng. Res. **46** (2008) 833.
27. *Kong, J. A., Han, Y. S., Han, T. J.,* Algae **17** (2002) 259.
28. *Kloareg, B., Quatrano, R. S.,* Mar. Biol. **26** (1988) 259.
29. *Michel, C., Macfarlane, G. T.,* J. Appl. Bacteriol. **80** (1996) 349.
30. *Jensen, A.,* Hydrobiologia **260** (1993) 15.
31. *Lloyd, T. A., Wyman, C. E.,* Bioresour. Technol. **96** (2005) 1967.
32. *Yeon, J. H., Seo, H. B., Choi, W. Y., Choi, W. S., Kang, D. H., Lee, H. Y., Jung, K. H.,* KSBB. **25** (2010) 283.