Thesis for the Degree of Master of Engineering

Optimization of Saccharification and Bioethanol Production by Simultaneous Saccharification and Fermentation (SSF) from Seaweed, *Saccharina japonica*

by

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Pukyong National University

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(해조류 다시마의 당화 최적화 및
동시당화발효를 이용한 바이오에탄올 생산)

Advisor: Prof. Sung-Koo Kim

by

Ji-Suk Jang

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CONTENTS

LIST OF FIGURES AND TABLE

해조류 다시마의 당화 최적화 및 동시당화발효를 이용한 바이오에탄올 생산

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요 약

산업혁명 이후 화석연료의 사용량은 급속도로 증가하여 현재 전세계 에너지 사용량의 약 86%에 달하고 있으며, 이러한 화석연료의 사용으로 인해 지구온난화 등의 환경 문제가 범세계적으로 부각되고 있다. 이로 인하여 재생 가능한 바이오 연료에 대한 관심이 증가하고 있으며, 바이오에탄올은 액체연료인 휘발유를 대체할 수 있는 유력한 대체 연료로서 세계적으로 그 생산량이 급증하고 있다. 해조류는 신재생에너지를 생산하기 위한 제 3 세대 바이오매스로 최근 관심이 집중되고 있다. 해조류를 바이오매스로 이용하여 바이오에탄올을 생산하기 위하여 해조류 탄수화물의 당화공정은 필수적이다.

본 연구에서는 해조류 다시마의 열가수분해 조건 최적화와 함께 해수분리균의 처리를 통한 당화 최적화 및 동시당화발효를 이용한 바이오에탄올 생산을 수행하였다. 묽은황산 및 공업용효소를 촉매로 이용한 열가수분해와 함께 해수로부터 직접 분리한 당화균인 *Bacillus sp.* JS-1 의 처리를 통하여 최종 당화 수율 69.1%을 얻을 수 있었다. *Bacillus sp.* JS-1 와 함께 에탄올 발효를 위한 4 가지 다른 효모인 *Pichia angophorae* KCTC 17574, *Pichia stipitis* KCTC 7228, *Saccharomyces serevisiae* KCCM 1129 그리고 *Pachysolen tannophilus* KCTC 7937 를 다시마 가수분해물에 각각 접종하여 동시당화발효를 수행 한 결과 0.39 g dcw/L 의 *Bacillus sp.* JS-1 과 0.45 g dcw/L 의 *Pichia angophorae* KCTC 17574 를 이용하였을 때 7.7 g/L 의 바이오에탄올을 생산하여 이론적 수율의 33.3%를 달성하는 것을 확인하였다.

이는 해조류 바이오매스로 이용한 바이오에탄올 생산공정의 효율적인 면에서 유용한 단위공정으로 이용될 수 있을 것으로 사료된다.

Ⅰ. INTRODUCTION

World faces the progressive depletion of its energy resources mainly based on non-renewable fuels (Lu *et al.,* 2009). The depletion of fossil fuel reserves, the unstable panorama of the petrol prices and increasing environmental and political pressures (Davis *et al.,* 2005) has encouraged the search of products originated from biomass as renewable sources of energy (Cazetta *et al.,* 2007; Davis *et al.* 2005). Moreover, renewable bioenergy has been introduced as an important contribution to future sustainable energy system (Berndes *et al.,* 2003). Among them, bioethanol from sugar based biomass is one of the energy carrier that can be used as a transportation fuel (Karakashev *et al.,* 2007), has traditionally been produced from starch or sugars containing feedstocks such as corn, wheat and sugarcane (Bothast *et al.,* 2005). However, due to the high prices of feedstocks that account for almost 40-75% of the total ethanol production and the competition with food, alternative feedstocks need for the ethanol production such as wastes or agricultural residues (lignocellulosic biomass) which include straw, wood and waste (Lu *et al.* 2009). However, lignocellulosic biomass contains lignin which cannot be easily degraded. Therefore, the biomass production is one of the major global concerns for energy sources (Berndes *et al.* 2003).

Seaweed is a third generation energy biomass with advantages such as no competition with food, fast growth and lignin-free composition. Seaweeds,

especially the species *Saccharina japonica* (Sea tangle, Dasima), *Undaria pinnatifida* (Sea mustard, Miyuk) and *Porphyra* species (Green laver, Gim) are extensively farmed in Asia, including China, Japan and Korea. The seaweed industry has an estimated total annual value of 5.5–6 billion US $, produced from 7.5–8 million tones of naturally growing and cultivated seaweed harvested worldwide. The main use is of seaweed as food products for human consumption, which generate approximately 5 billion US $ per year, with the remainder generated from extracted hydrocolloids, fertilizers and animal feed additives (Adams *et al.,* 2009; McHugh 2003). *Saccharina japonica* which mass cultivation is the brown seaweed available in Korea, was used as a biomass for bioethanol production in this study. Brown seaweed has a high contents of easily degradable carbohydrates, making them a potential substrate for the production of liquid fuels (Horn *et al.,* 2000). Brown seaweed has a high contents of alginate, laminaran and mannitol (Horn *et al.,* 2000), and the main organic component is alginate which is as high as 50% (Tang *et al.,* 2009). Alginate is an unbranched polysaccharide produced by *Psedomonas* species, *Azobactor vinelandii*, and several species of brown seaweed. Alginate is composed by D-mannuronate and its C5-epimer, L-guluronate, which are linked by ß-(1→4) glycosidic linkages and a major structural component of cell wall and intracellular matrix of brown seaweeds. The L-guluronate is probably derived from D-mannuronate by the action of C5-epimerase (Choi *et al.,* 2009; Kloareg *et al.,* 1988). And laminaran is a linear polysaccharide of ß-(1→3)-D-glucose in which the chain terminates with D-mannitol with low

levels of branch by ß-(1→6)-glucosidic linkages (Horn *et al.* 2000; Myklestad 1978). Mannitol, the sugar alcohol corresponding to mannose, is also one of the main sugar components of brown seaweeds (Horn *et al.* 2000).

Saccharification of seaweed is an essential unit operation for the bioethanol fermentation and has been widely investigated in recent years. Various physical, chemical and biological pretreatment approaches have been proven to increase the efficiency of saccharification (Lu *et al.* 2009). Chemical hydrolysis of biomass with dilute sulfuric acid has been long recognized as a critical step for removing the hemicellulosic fraction from the lignocellulosic substrate to economize the biological conversion of cellulosic biomass to ethanol (Kuhad *et al.,* 2010). There have been two general methods of the saccharification from alginate. Firstly, oligosaccharides can be prepared by partial acid and alkali hydrolysis. Secondly, alginate lyase obtained from various microorganisms can be used (Choi *et al.* 2009). Thus, the isolation of specific microorganisms is essential (Tang *et al.* 2009) for the efficient saccharification of seaweed.

Generally, there are four steps of bioethanol production process from biomass; pretreatment, enzymatic hydrolysis, fermentation and distillation (Sun *et al.,* 2002; Tomas-Pejo *et al.,* 2009). Combined saccharification and fermentation processes is called simultaneous saccharification and fermentation (SSF) (Na *et al.,* 2008). It has been shown that higher ethanol yields could be obtained in SSF processes compared with separate hydrolysis and fermentation (SHF) (Alfani *et al.,* 2000; Stenberg *et al.,* 2000; Tomas-Pejo *et al.* 2009). Simultaneous saccharification and

fermentation (SSF) is an efficient process for the bioethanol production by the reduction of unit operations (Wingren *et al.,* 2003).

In this study, thermal acid hydrolysis was carried out for the pretreatment of seaweed slurry. SSF was also performed using the isolated marine bacteria, *Bacillus sp*. JS-1 for the saccharification and various yeasts for the bioethanol fermentation.

Ⅱ. MATERIALS AND METHODS

1. Raw material

Saccharina japonica (sea tangle) was obtained from Gijang fisheries market in Busan, Korea. Seaweed was dried with sunlight or hot-air and then ground by hammer mill. The powder was screened through 20 mesh sieve prior to the pretreatment. Composition and proximate analysis of *Saccharina japonica* and other seaweeds were determined by the Feed and Foods Nutrition Research Center at Pukyong National University in Korea, according to the procedures recommended by the National Exposure Research Laboratory (NERL, USA) (Wei *et al.,* 2009).

2. Microorganisms, medium and the preparation of inoculum

Isolated marine bacteria, *Bacillus sp.* JS-1 was used for the saccharification of *Saccharina japonica*. YPD broth (yeast extract 10.0 g/L, peptone 20.0 g/L, dextrose 20.0 g/L) was sterilized at 121℃ for 15 min and used medium for the *Bacillus sp.* JS-1 culture. The seed culture (5 ml YPD medium in 13 x 100 mm test tube) was

prepared with a single colony of *Bacillus sp.* JS-1 from YPDA plate and incubated in a rotary shaker at 30℃ for 12 hrs. The second culture (70 ml YPD medium in 250 ml Erlenmeyer flask) was prepared with the seed culture (5%, v/v) and incubated in a rotary shaker at 30℃ and 200 rpm for 13 hrs (OD_{600} 0.8-0.9). The stock vials were prepared and maintained at -80°C in YPD with 20% glycerol.

Pichia angophorae KCTC 17574, *Pichia stipitis* KCTC 7228, *Saccharomyces serevisiae* KCCM 1129 and *Pachysolen tannophilus* KCTC 7937 were used for the ethanol fermentations. YPD broth was used for the medium for the yeasts. A single colony of yeasts from YPDA plate was used for the preparation of seed culture and the culture was incubated in a rotary shaker at 30℃ for 12 hrs. The second cultures (25 ml YPD medium in 100 ml Erlenmeyer flask) were prepared with the seed cultures (5%, v/v) and the cultures of *Pichia angophorae* KCTC 17574, *Pichia stipitis* KCTC 7228, *Saccharomyces serevisiae* KCCM 1129 and *Pachysolen tannophilus* KCTC 7937 were incubated in a rotary shaker at 30℃ and 200 rpm for 13, 13, 10 and 8 hrs (OD_{600} 0.8-0.9), respectively. The stock vials of yeasts were prepared and maintained at -80°C in YPD with 20% glycerol.

3. Analytical methods

To determine cell density, OD_{600} of cell suspension was measured using the UV-Vis spectrophotometer and converted to dry cell weight using standard curve. Reducing sugar concentrations were determined by the 3, 5-dinitrosalicyclic acid

(DNS) method with glucose (Sigma Chemical Co. USA) as the standard (Dubois *et al.,* 1956). Viscosity was measured three times using the Brookfield viscometer (BROOKFIELD DV-Ⅲ Rheometer v3.1, Brookfield Eng. Inc., USA) connected with spindle No. ULA, SC4-18, SC4-34 at temperature of 30℃. Salinity was measured by salinometer (Salinity Refractometer, ATAGO. Inc., Japan). Ethanol concentrations were measured by GC (HP 5890 series Ⅱ, Hewlett Packard. Inc., USA) using a flame ionization detector (FID) with a HP-FFAP column (Cross-Linked PEG-TPA; length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm) . Nitrogen was used as the carrier gas with a flow rate of 0.6 ml/min. The injection and detector temperature were controlled at 150℃ and 200℃, respectively. The increasing temperature condition was 45℃ (2 min) / (1℃/min) / 50℃ (1 min) / (20℃/min) / 90℃ (1 min) / (30℃/min) / 150℃ (1 min). Mannitol, fucose, xylose, glucose, galactose, alginate, laminaran, fucoidan and organic acids from samples of SSF were filtrated through 0.2 μm and quantified by HPLC (Agilent 1100 Series, Agilent. Inc., USA) equipped with an Agilent G1362A refractive index detector (RID) detector. Biorad Aminex HPX-87H column (300 x 7.8 mm) and Supelguard C610H column (50 x 4.6 mm) using filtered and degassed 50 mm H_2SO_4 as eluent, 0.6 ml/min of flow rate, and 65℃ of column temperature.

4. Thermal acid hydrolysis

Two different thermal acid hydrolysis procedures were carried out with and without enzyme α-amylase (Termamyl 120 L, Novozymes. Inc., Denmark). The thermal acid hydrolysis without the Termamyl 120 L was carried out with 100 ml of seaweed slurry (10%, w/v) by the addition of 0.2% (w/v, 0.04 M) H_2SO_4 in 250 ml Erlenmeyer flask. The thermal acid hydrolysis with the Termamyl 120 L was carried out with the addition of 1 ml of Termamyl 120 L (1.5 KNU/ml) and 0.2% (w/v, 0.04 M) H_2SO_4 to 100 ml of seaweed slurry (10%, w/v) in 250 ml Erlenmeyer flask. The mixture was autoclaved at 121℃ for 60 min. After completion of the autoclave, samples were allowed to cool down to room temperature and neutralized by 5 N NaOH. Each sample was replicated three times.

5. Saccharification of *Saccharina japonica* by isolated marine bacteria, *Bacillus sp.* JS-1

For effective saccharification of *Saccharina japonica*, the treatment of the marine bacteria, *Bacillus sp.* JS-1 was carried out. After the thermal acid hydrolysis with 0.2% (w/v, 0.04 M) H_2SO_4 at 121℃ for 60 min, the seaweed slurry was neutralized with 5N NaOH and 10% (v/v, 1 g dcw/L) *Bacillus sp.* JS-1 were inoculated to the slurry. The saccharification was carried out at 30℃, 200 rpm for 7.5 days. Reducing sugar concentrations and viscosities were measured.

6. Simultaneous Saccharification and Fermentation (SSF)

After the thermal acid hydrolysis with 0.2% (w/v, 0.04 M) H_2SO_4 at 121℃ for 60 min, the seaweed slurry was neutralized with 5N NaOH. 4 % (v/v, 0.39 g dcw/L) *Bacillus sp.* JS-1 and 4% (v/v, 0.45 g dcw/L) yeasts were inoculated to the thermal acid hydrolysate for SSF. The mixtures were fermented at 30℃, 200 rpm for 136 hrs. Reducing sugar and ethanol concentrations were measured at the interval of 12 hr. The samples for the analysis were centrifuged at 15,000 rpm for 10 min and the supernatants were frozen at -20℃ until analysis.

Ⅲ. RESULTS AND DISCUSSION

1. Carbohydrate contents of seaweeds

The composition and proximate analysis of several seaweeds were listed in Table 1. *Saccharina japonica*, *Sargassum fulvellum*, *Undaria pinnatifida* and *Hizikia fusiforme* are brown seaweeds. *Enteromorpha linza* is green seaweed and *Gelidium amansii* is red seaweed. *Saccharina japonica*, *Sargassum fulvellum*, *Undaria pinnatifida* and *Hizikia fusiforme* had total carbohydrate 44.5-66.0% (w/w), crude ash 21.8-35.1% (w/w), crude protein 10.6-19.9% (w/w) and crude lipid 0.4-1.8% (w/w). *Enteromorpha linza* had the lowest carbohydrate contents of 37.4% (w/w) and *Gelidium amansii* had the highest carbohydrate content of 74.4% (w/w). Biomass with high carbohydrate content is essential for the bioenergy production. *Gelidium amansii* shows highest carbohydrate content. However, *Gelidium amansii* grows in a tropical climate, thus, this species is hard to culture in Korea and the mariculture system has not been established. Therefore, *Saccharina japonica* with 66.0% (w/w) of total carbohydrate content and well established mariculture system was selected for the bioethanol production in this study. *Saccharina japonica* had 6.3% (w/w) of crude fiber, which are cellulose and lingnin among 66.0% (w/w) of total carbohydrate.

Table 1. Compositions of seaweeds

Seaweed	Composition (%, w/w)			
	Crude protein	Crude lipid	Crude ash	Total carbohydrate (fiber)
Saccharina japonica	10.6	1.6	21.8	66.0 (6.3)
Sargassum fulvellum	19.9	0.5	35.1	44.5 (3.5)
Undaria pinnatifida	18.3	1.8	28.0	52.0 (3.6)
Hizikia fusiforme	13.9	0.4	26.6	59.0 (4.2)
Enteromorpha linza	31.6	1.8	29.2	37.4 (2.4)
Gelidium amansii	18.3	0.0	7.4	74.4 (11.6)

Compositions of seaweeds were determined by the Feed and Foods Nutrition Research Center at Pukyong National University in Korea according to the procedures recommended by the National Exposure Research Laboratory (NERL, USA)

2. Thermal acid hydrolysis

Two thermal acid hydrolysis methods were carried out. 0.2% (w/v, 0.04 M) H_2SO_4 with and without Termamyl 120 L was added to 10% (w/v) seaweed slurry for the thermal acid hydrolysis. Reducing sugar concentrations of thermal acid hydrolysis were shown in Fig. 1. Reducing sugar of 14.5±2.1 g/L was produced with 0.2% (w/v, 0.04 M) H_2SO_4 as a catalyst. Reducing sugar of 20.6±1.9 g/L was produced with 1% (1.5 KNU/ml) Termamyl 120 L and 0.2% (w/v, 0.04 M) H_2SO_4 treatment. Termamyl 120 L is an endoamylase produced by a genetically modified strain of *Bacillus licheniformis*. Termamyl 120 L hydrolyzes α-(1→4)-glucosidic linkages in amylose and amylopectin with advantages of the extreme heat stability (121℃) and broad pH tolerance with the actions of liquefaction of carbohydrate. Termamyl 120 L is used for adjunct liquefaction in the brewing industry. The addition of Termamyl 120 L degraded the carbohydrate of seaweed, thus, the reducing sugar concentration of seaweed slurry increased by thermal acid hydrolysis with Termamyl 120 L.

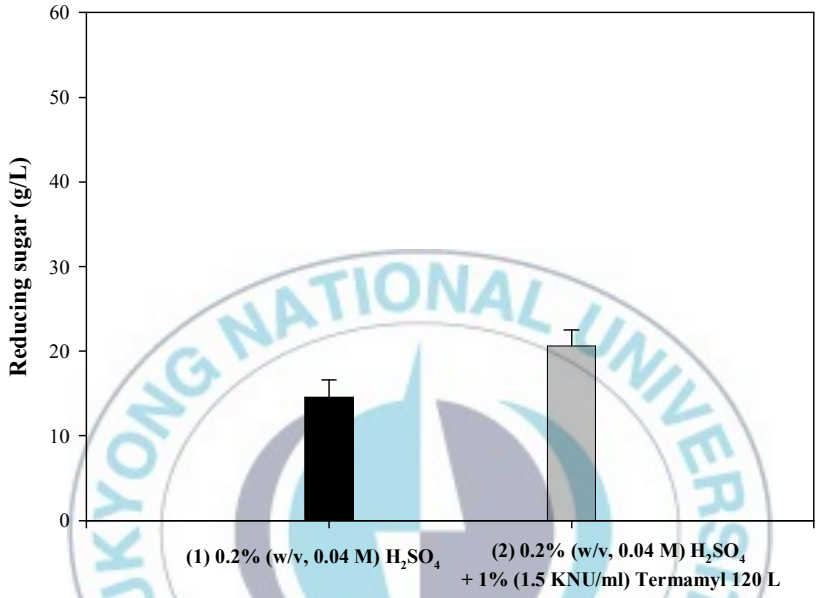

Fig. 1. Reducing sugar concentrations by thermal acid hydrolysis.
(1) Thermal acid hydrolysis of 10% (w/v) *Saccharina japonica* slurry at 121℃ for 60 min with 0.2% (w/v, 0.04 M) H_2SO_4 and (2) Thermal acid/enzyme hydrolysis of 10% (w/v) *Saccharina japonica* slurry with 1% (1.5 KNU/ml) Termamyl 120 L and 0.2% (w/v, 0.04 M) H_2SO_4.

3. Saccharification of *Saccharina japonica* by isolated marine bacteria, *Bacillus sp.* JS-1

Saccharification of *Saccharina japonica* by *Bacillus sp.* JS-1 was carried out for the increase of reducing sugar concentration. High reducing sugar concentration and low viscosity from the saccharification of *Saccharina japonica* by the *Bacillus sp.* JS-1 were obtained as shown in Fig. 2. Reducing sugar concentration was increased and viscosity was decreased by the saccharification using *Bacillus sp.* JS-1. Fig. 2(A) shows that the reducing sugar concentration of 13.9±0.8 g/L with the thermal acid hydrolysis increased to 45.6±6.8 g/L with the treatment of *Bacillus sp.* JS-1. The untreated slurry as a control showed viscosity of 117.0 cp and the thermal acid hydrolysis of the seaweed slurry decreased the viscosity to 49.1±1.3 cp. The *Bacillus sp.* JS-1 treatment to the seaweed slurry acid hydrolysate showed further decrease of viscosity to 27.7±13.7 cp as shown in Fig. 2(B).

Fig. 3 shows the reducing sugar concentrations of the seaweed slurry acid hydrolysate by the treatment of *Bacillus sp.* JS-1. The reducing sugar concentrations increased linearly with time. This indicates that the mode of degrading enzyme action by *Bacillus sp.* JS-1 is the exo-lyase for the carbohydrate of the seaweed slurry. Reducing sugar concentrations of the seaweed slurry by *Bacillus sp.* JS-1 treatment could reached to 4 times higher than that of thermal acid hydrolysis treatment.

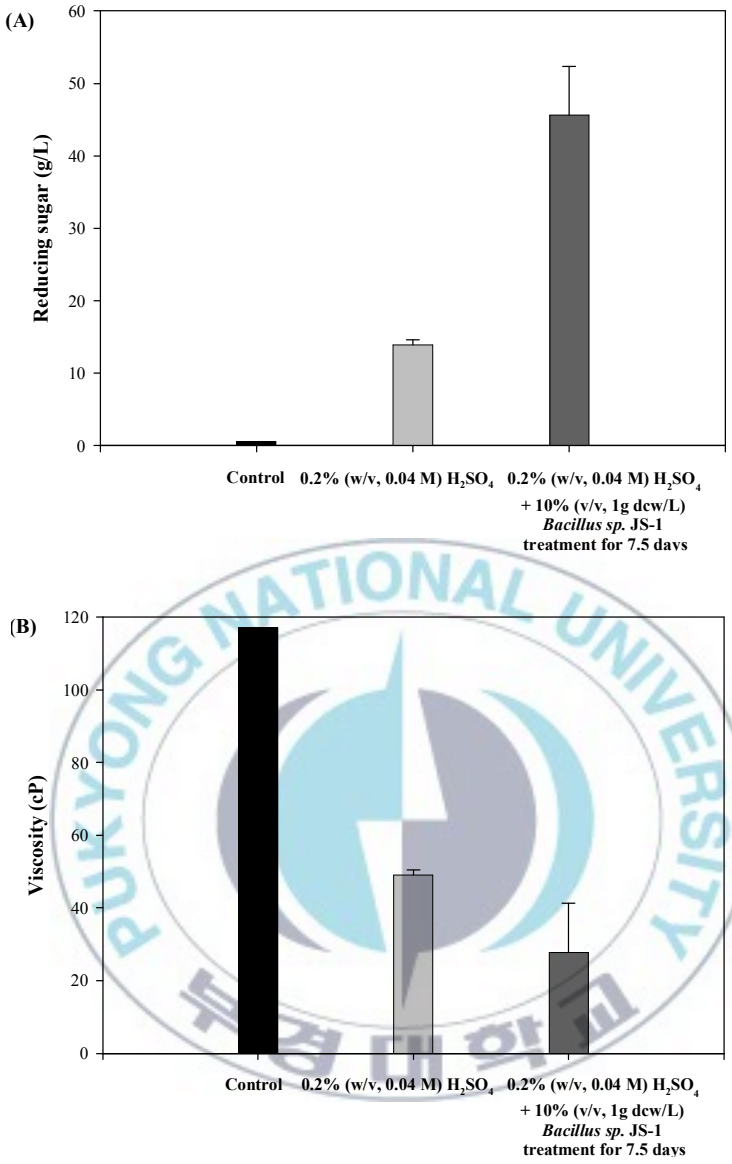

Fig. 2. Reducing sugar concentrations and viscosities of seaweed slurry (10%, w/v) by thermal acid hydrolysis and the treatment of the isolated marine bacteria, *Bacillus sp.* JS-1.
(A) changes of reducing sugar concentrations and (B) changes of viscosities.

Bacillus sp. JS-1 was selected as the best activity of four isolated bacteria stains from seawater and identified as *Bacillus sp.* (100%) by 16S ribosomal RNA sequence and BLASTN analysis. The isolated marine bacterium was named as *Bacillus sp.* JS-1. This indicates that *Bacillus sp.* can be adapted to wide range of salinity. Alginate, fucoidan and laminaran and other polysaccharides from seaweed could be degraded by the isolated marine *Bacillus sp.* JS-1.

The summary of thermal acid hydrolysis for the pretreatments and the *Bacillus sp.* JS-1 treatment for the saccharification of the carbohydrate in *Saccharina japonica* was listed in Table 2. Condition 1 with 0.2% (w/v, 0.04 M) H_2SO_4 only showed 14.5±2.1 g/L of reducing sugar concentration with 21% yield and Condition 2 with 0.2% (w/v, 0.04 M) H_2SO_4 and 1% (1.5 KNU/ml) Termamyl 120 L showed 20.6±1.9 g/L of reducing sugar concentration with 31.2% yield from *Saccharina japonica*. Condition 3 with *Bacillus sp.* JS-1 showed 45.6±5.0 g/L of reducing sugar concentration with 69.1% yield from *Saccharina japonica.*

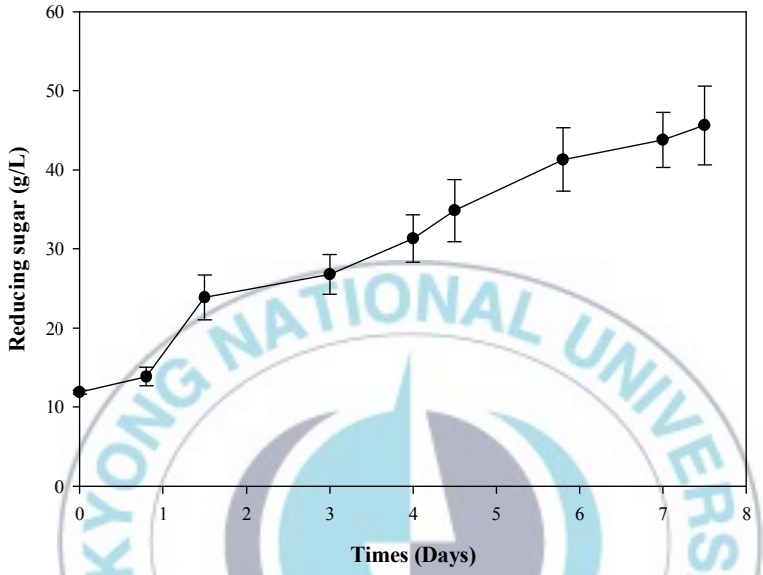

Fig. 3. Changes of reducing sugar concentrations of seaweed slurry (10%, w/v) by the treatment of *Bacillus sp.* JS-1 after the thermal acid hydrolysis.

Table 2. Summary of thermal acid/enzyme hydrolysis and biological saccharification by *Bacillus sp.* JS-1

Experiments	Conditions	Reducing sugar concentrations	Yields of saccharification form carbohydrate of raw material
Condition 1	10% (w/v) *S. japonica* 0.2% (w/v, 0.04 M) H_2SO_4 121℃, 60 min	14.5±2.1 g/L	21.0%
Condition 2	10% (w/v) *S. japonica* 1% (1.5 KNU/ml) Termamyl 120 L 0.2% (w/v, 0.04 M) H_2SO_4 121℃, 60 min	20.6±1.9 g/L	31.2%
Condition 3	10% (w/v) *S. japonica* 0.2% (w/v, 0.04 M) H_2SO_4 121℃, 60 min 10% (v/v, 1 g dcw/L) *Bacillus sp.* JS-1 30℃, 200 rpm, 7.5 days	45.6±5.0 g/L	69.1%

4. Simultaneous Saccharification and Fermentation (SSF)

Simultaneous Saccharification and Fermentation (SSF) was carried out by the addition of the 0.39 g dcw/L of *Bacillus sp.* JS-1 and 0.45 g dcw/L of yeasts to thermal acid hydrolyzed slurry. Ethanol productions were determined as shown in Fig. 4. The highest ethanol production reached 7.7 g/L (1% (v/v)) by the yeast, *P. angophorae* after 64.2 hrs of fermentation. And ethanol concentration was reduced by further fermentation due to the evaporation of ethanol. *P. stipitis, S. cerevisiae* and *P. tannophilus* produced little amount of ethanol reaching to 0.9-1.8 g/L of ethanol concentrations. .

In order to reduce the energy consumption of the distillation step, the ethanol concentration should be higher than 4% (v/v) (Li *et al.,* 2009; Tomas-Pejo *et al.* 2009; Wingren *et al.* 2003; Zacchi *et al.,* 1989). However, the highest ethanol concentration was 1% (v/v) in this study. *P. angophorae* could use fermentable monosugars of glucose, mannitol, xylose from brown seaweed (Horn *et al.* 2000). Brown seaweed is composed of alginate, mannitol, laminaran, fucoidan in large part of carbohydrate components (Horn 2000; Horn *et al.* 2000; Kloareg *et al.* 1988). Therefore, mixed cultures with various yeasts which can use various sugars from brown seaweed should be performed for the production of high ethanol concentration from seaweed. Further studies on the fermentation of various sugars from the seaweed are now carried out.

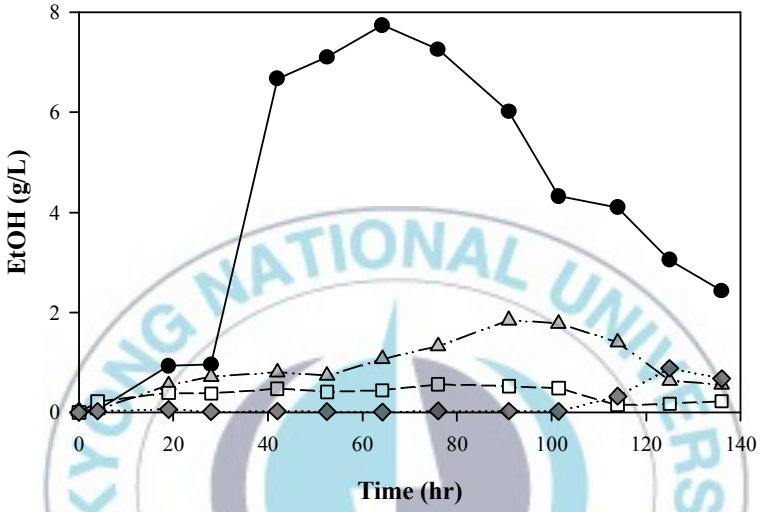

Fig. 4. Bioethanol production from *Saccharina japonica* by SSF with 4 different yeasts
(●) *Pichia angophorae* KCTC 17574, (▲) *Pichia stipitis* KCTC 7228, (□) *Saccharomyces serevisiae* KCCM 1129 and (◆) *Pachysolen tannophilus* KCTC 7937

The ethanol production by SSF with *Pichia angophorae* KCTC 17574 was shown in Fig. 5. Ethanol concentration was rapidly increased during 28-42 hr. More ethanol was produced slowly for next 22 hrs (42-64 hr). The maximum ethanol concentration reached to 7.7 g/L. Sixty-five ‰ of salinity was decreased to 45‰ during the decreasing period (64-102 hr) of ethanol and the salinity increased during the period of ethanol evaporation. During ethanol fermentation, pH of fermentation broth was 7.0 after the neutralization by the addition of 5N NaOH and then decreased to 4.3 by the acidogenesis. Fig. 5 shows acetic acid production as typical fermentation by-products from glucose. By-products are not only acetic acid but also propionic acid and butyric acid including ethanol (Horn 2000; Horn *et al.,* 2001). Acetic acid concentration was increased constantly to 7.2 g/L during SSF. To optimize ethanol production with inoculums size, temperature, pH and agitation and the removal of process inhibitors such as acetic acid, salt, HMF and furfural is required.

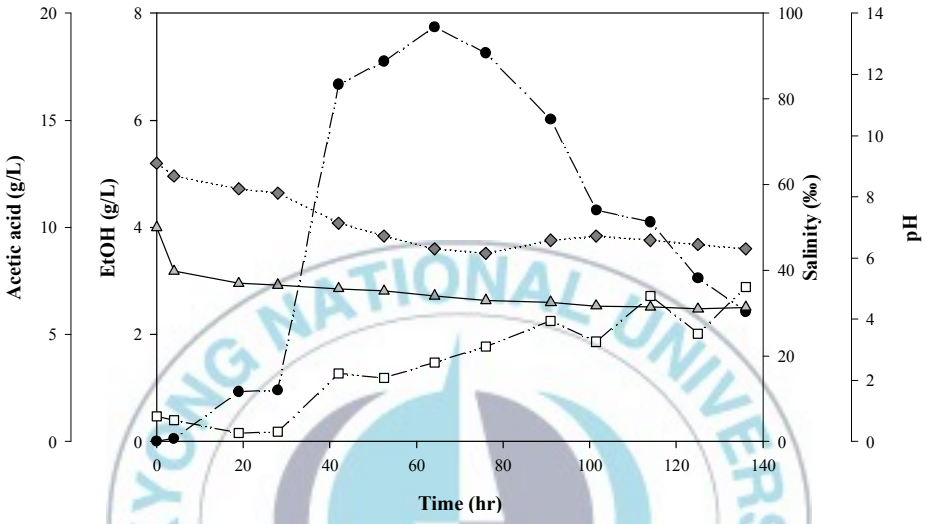

Fig. 5. Bioethanol production by SSF with *Bacillus sp.* JS-1 and *Pichia angophorea*

(●) Ethanol concentration (g/L), (◆) Salinity (‰), (□) Acetic acid concentration (g/L) and (▲) pH

Ⅳ. CONCLUSION

Optimization of saccharification process was carried out with thermal acid hydrolysis and/or the treatment of isolated marine bacteria, *Bacillus sp.* JS-1. Thermal acid hydrolysis showed 14.5±2.1 g/L of reducing sugar concentration with 21% yield from carbohydrate of *Saccharina japonica*. Thermal acid hydrolysis with Termamyl 120 L showed 20.6±1.9 g/L of reducing sugar concentration with 31.2% yield from the carbohydrate of *Saccharina japonica*. Thermal acid hydrolysis and *Bacillus sp.* JS-1 treatment showed 45.6±5.0 g/L of reducing sugar concentration with 69.1% yield from the carbohydrate of *Saccharina japonica*.

Mannitol is not reducing sugar due to lack of reducing hydroxyl group (C1-OH) so it cannot be detected by DNS method. Therefore, yield of saccharification is expected to be higher than 69.1% when mannitol is quantified using HPLC.

Simultaneous saccharification and fermentation (SSF) for bioethanol production showed 33.3% yield with theoretical yield with 7.7 g/L of ethanol production when the *Bacillus sp.* JS-1 for the saccharification and the yeast, *Pichia angophorae* for ethanol production were used.

This can be applied for useful bioethanol production processes and showed possibility of bioethanol production as alternative liquid fuel from seaweed.

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실험진행에 있어 중요한 포인트를 콕콕 집어주시어 제가 열심히 공부하도록 유도해주신 유기방의 박남규 교수님과 실험적인 면에서 어떻게 해야 할지 몰라 당황하던 저에게 친절과 미소로 꼼꼼히 가르쳐주신 정귀택 교수님께도 감사를 드립니다. 그리고 학부시절 지도교수님이셨던 홍용기 교수님, 해조류에 대해서 많이 알게 해주셨습니다. 감사합니다. 복도에서 가끔 뵐 때 고품격의 즐거운 유머로 힘을 주신 김중균 교수님과 수업시간에 집중할 수밖에 없었던 너무 멋있으신 공인수 교수님께도 감사의 말씀을 드립니다. 옆방의 이형호 교수님, 아직도 수업시간에 말씀해주신 우주와 생명의 연결고리가 생각이 납니다. 수업이 아니라 진리를 듣는 것 같습니다.

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