J. Microbiol. Biotechnol. (2012), **22**(1), 107–113
http://dx.doi.org/10.4014/jmb.1106.06019
First published online October 15, 2011

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Development of a Practical and Cost-Effective Medium for Bioethanol Production from the Seaweed Hydrolysate in Surface-Aerated Fermentor by Repeated-Batch Operation

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Received: June 9, 2011 / Revised: October 7, 2011 / Accepted: October 8, 2011

To develop a practical and cost-effective medium for bioethanol production from the hydrolysate of seaweed *Sargassum sagamianum*, we investigated the feasibility and performance of bioethanol production in CSL (corn-steep liquor)-containing medium, where yeast *Pichia stipitis* was used and the repeated batch was carried out in a surface-aerated fermentor. The optimal medium replacement time during the repeated operation was determined to be 36 h, and the surface aeration rates were 30 and 100 ml/min. Under these conditions, the repeated-batch operation was successfully carried out for 6 runs (216 h), in which the maximum bioethanol concentrations reached about 11–12 g/l at each batch operation. These results demonstrated that bioethanol production could be carried out repeatedly and steadily for 216 h. In these experiments, the total cumulative bioethanol production was 57.9 g and 58.0 g when the surface aeration rates were 30 ml/min and 100 ml/min, respectively. In addition, the bioethanol yields were 0.43 (about 84% of theoretical value) and 0.44 (about 86% of theoretical value) when the surface aeration rates were 30 ml/min and 100 ml/min, respectively. CSL was successfully used as a medium ingredient for the bioethanol production from the hydrolysate of seaweed *Sargassum sagamianum*, indicating that this medium may be practical and cost-effective for bioethanol production.

Keywords: Corn-steep liquor, bioethanol, seaweed *Sargassum sagamianum*, repeated-batch fermentor culture, surface aeration

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Seaweed was recently identified as a potential alternative renewable biomass for bioethanol production [4, 11, 29], because bioethanol production from sugar/starch crops through traditional production technologies and bioethanol production from lignocellulosic biomass have inevitable drawbacks such as unsustainable supply of crops, difficulty in complete hydrolysis of polysaccharides, and low content of fermentable sugar. Specifically, it has been suggested that the seaweed *Laminaria*, *Saccorhiza*, and *Alaria* groups, belonging to brown seaweed [1, 8, 9, 21], and *Gelidium amansii*, belonging to red seaweed, can be used as potential resources for bioconversion to ethanol [29, 33].

In our previous study [31], the hydrolysate of seaweed *Sargassum sagamianum* (*S. sagamianum*) was shown to consist mainly of some monosaccharides such as glucose, xylose, fructose, and mannose. In addition, throughout repeated-batch culture using the hydrolysate of seaweed *S. sagamianum*, we demonstrated that the bioethanol production process could be carried out repeatedly and steadily in flask and fermentor cultures using *Pichia stipitis* (*P. stipitis*). Thus, this approach is a practical strategy for commercial bioethanol production from seaweed, where the bioethanol production can be carried out in a surfaced-aerated fermentor culture to convert C5 monosaccharides (*e.g.*, xylose) into bioethanol by finely controlling aeration [31, 32]. Although this previous study was conducted in a lab-scale fermentor, we found that bioethanol production from the hydrolysate of seaweed *S. sagamianum* could be reliably repeated when a surface-aerated fermentor was used for microaeration. Thus, we expect that this process will be applicable to larger scale bioethanol production.

However, in this previous study, YPH medium [yeast extract (10 g/l) and peptone (20 g/l) were used for bioethanol production from the hydrolysate of seaweed *S. sagamianum*] [31, 32]. Unfortunately, yeast extract and

peptone, which were used as a vitamin and N-source source, respectively, are very expensive, particularly for bioethanol production. Thus, alternative, low-cost ingredients are needed for practical bioethanol production from the hydrolysate of seaweed *S. sagamianum*. Therefore, in this study, we investigated the potential of using corn-steep liquor (CSL) as a substitute for yeast extract and peptone. CSL is a by-product of corn wet-milling, and an important constituent in microbial growth media as an excellent source of organic nitrogen. In several previous studies, CSL was investigated as a potential N-source in culture medium for industrial applications [5, 13, 15, 16, 22, 24], particularly for ethanol production [2, 12, 14, 19, 26–28].

In this study, repeated-batch operation in the surface-aerated fermentor using *P. stipitis* was conducted as previously described [30, 31] and we investigated whether both the hydrolysate of seaweed and CSL could be used for bioethanol production. Thus, the aim of this study was to examine the feasibility and the performance of bioethanol production from medium containing the hydrolysate of seaweed *S. sagamianum* and CSL. The use of CSL as a medium ingredient is expected to make bioethanol production more practical and cost-effective.

The yeast strain used in this study was *P. stipitis* CBS 7126. The yeast extract and peptone were purchased from Becton Dickinson and Co. (Sparks, MD, USA). CSL was purchased from Corn Product Korea (Gyeonggi-Do, Republic of Korea). Glucose, xylose, *n*-butanol, acetonitrile, and sulfuric acid were from Daejung Chemical Co. (Gyeonggi-Do, Republic of Korea). Phosphoric acid and ammonia water were from Wako Chemical Co. (Osaka, Japan) and Junsei Chemical Co. (Tokyo, Japan), respectively. Dinitrosalicylic acid and α-naphtol were obtained from Samchun Chemical Co. (Seoul, Republic of Korea) and Sigma-Aldrich (St. Louis, MO, USA), respectively.

A high-temperature liquefying system (Ilshin, Republic of Korea) was used to efficiently hydrolyze the seaweed *S. sagamianum* [20]. In these experiments, 10 g of dry seaweed *S. sagamianum* was added to 100 ml of distilled water at 200°C and subjected to 15 MPa for 15 min. The other conditions used during this process have been previously described in detail [6, 31, 32]. The seed culture was performed in a 250 ml Erlenmeyer flask with 50 ml of YPD medium (yeast extract, 10 g/l; peptone, 20 g/l; glucose, 20 g/l) in a shaking incubator at 30°C and 150 rpm. The fermentor culture was performed in a 2.5 l jar fermentor (KoBiotech, Republic of Korea) with an initial working volume of 880 ml, which was prepared by mixing 800 ml of CSH medium [CSL, 20 ml/l; $(NH_4)_2SO_4$, 1.2 g/l; KH_2PO_4, 2.4 g/l; and $MgSO_4 \cdot 7H_2O$, 1.2 g/l were dissolved in the hydrolysate of seaweed *S. sagamianum*] [17, 25] and 80 ml of seed culture. During fermentation, the temperature, pH, and agitation speed were maintained at 30°C, 5.0, and 200 rpm, respectively. In the repeated-batch culture, 800 ml of culture broth was withdrawn every 36 or 48 h, and 800 ml of new CSH medium was added. The pH was maintained by adding a solution of phosphoric acid [10% (v/v)] or ammonia water, as necessary. Surface aeration was carried out using silicon tubing (inside diameter = 3.1 mm). A tube (L = 6.7 cm) was inserted from the head plate into the inner headspace of the fermentor vessel (inside diameter = 14.2 cm). When agitation was not carried out, the distance from the surface of the medium to the end of the tubing was approximately 7 cm. The air supply rate was finely controlled using an air-flow meter (Cole-Parmer, USA).

Cell growth was assessed by measuring the optical density at 600 nm using a spectrophotometer, total reducing sugar was measured using the dinitrosalicylic acid (DNS) method [3], and ethanol concentration was measured by gas chromatography as described previously [31, 32]. The monosaccharides were analyzed *via* thin-layer chromatography (TLC) using a 20 × 10 cm Partisil K5F (Whatman) as the TLC plate and acetonitrile solution [acetonitrile:water = 85:15 (v/v)] as the mobile phase, with a sample loading volume of 1.0 µl. To visualize the bands, TLC plates were soaked in 0.5% α-naphtol and 5% H_2SO_4 in ethanol and then dried in an oven at 80°C for 15 min. The amount of glucose and xylose in the culture broth were quantitatively determined *via* TLC using the method described by Robyt and Mukerjea [23]. Using this approach, the intensity and density of the glucose, isomaltodextrin, and maltodextrin on the TLC plate was the same based on weight, and the density was linearly proportional to the weight of the sugar compound. Glucose and xylose standards were developed on the same TLC plate. The bands in the scanned images were converted to peaks using the AlphaEase FC software (Alpha Innotech, USA), and their quantities were calculated from the standard curves of glucose and xylose.

The hydrolysate of seaweed *S. sagamianum* consisted of different monosaccharides including glucose [65.5 (%, wt)], xylose [19.4 (%, wt)], fructose [7.4 (%, wt)], and mannose [2.8 (%wt)] [31]. Fortunately, the monosaccharide content in the polysaccharides of the hydrolysate of seaweed *S. sagamianum* consisted of 85.0 (%, wt) glucose and xylose, which can readily be converted to ethanol. In a previous report, we investigated the effect of the medium replacement time during the repeated-batch operation [31]. When YPH medium was used, a medium replacement time of 48 h was determined to be optimal for bioethanol production from the hydrolysate of seaweed *S. sagamianum*. Since CSL was used as a substitute for the yeast extract and peptone in this study, the optimal replacement time for repeated-batch operation was examined and compared with the optimal time determined for the yeast extract and peptone (Fig. 1). As shown in Fig. 1C and 1F, a medium replacement time of 36 h was determined to be the optimal time for the CSH

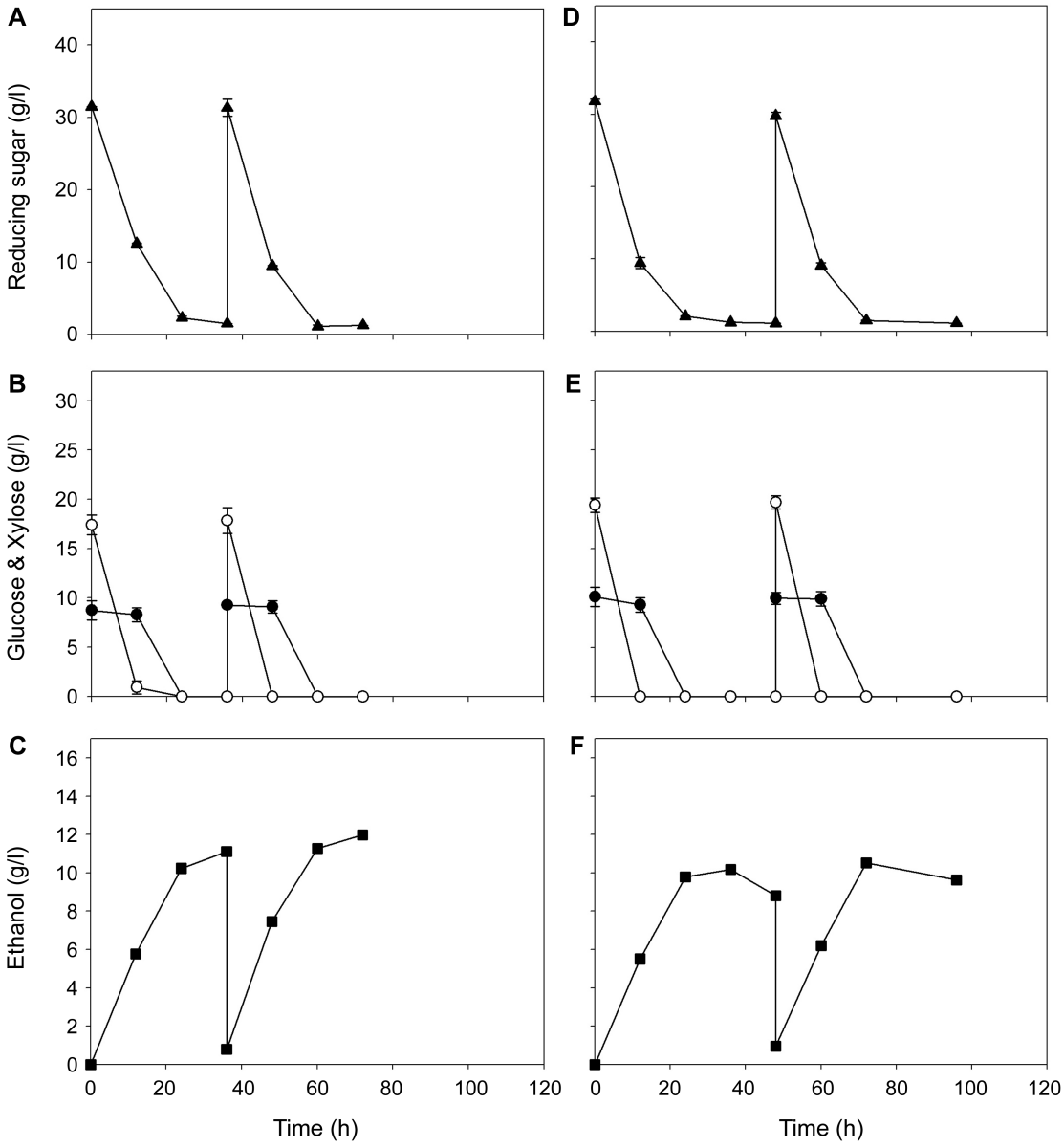

Fig. 1. Effect of medium replacement time during repeated-batch operation. The medium was replaced every 36 (**A**, **B**, **C**) and 48 h (**D**, **E**, **F**), respectively.

Air was supplied into the headspace of the fermentor at a rate of 100 ml/min. (**A**, **D**) Reducing sugar concentration in the culture supernatant. (**B**, **E**) Glucose (○) and xylose (●) concentrations in culture supernatant. (**C**, **F**) Bioethanol production. All measurements were conducted three times (n=3) using the same sample, and the average and standard deviations were calculated.

medium in terms of bioethanol production, which was shorter than the 48 h previously determined for the YPH medium [31]. Bioethanol production decreased after an elapsed time of 36 h owing to bioethanol consumption by *P. stipitis* (Fig. 1F). Therefore, a medium replacement time of 36 h was used in all subsequent experiments. In addition, we found that when the CSH medium was used, the rates of cell growth and sugar consumption increased when compared with the rates obtained when YPH medium was used. Furthermore, glucose and xylose were completely consumed at the medium replacement times of 36 h and 48 h (Fig. 1B and 1E). Meanwhile, Amartey and Jeffries [2] and Maddipati *et al.* [19] reported that the rates of cell growth, carbon source consumption, and ethanol production increased when CSL was used in the medium for ethanol production. In addition, Hull *et al.* [10] demonstrated that CSL consisted of carbohydrates, amino acids, polypeptides, fatty acids, other organic compounds, hydrolytic enzymes, trace metals, and inorganic ions. It was deduced that the use of CSL in the medium probably led to increased rates of cell growth, sugar consumption, and ethanol production, and thereby shortened the medium replacement time.

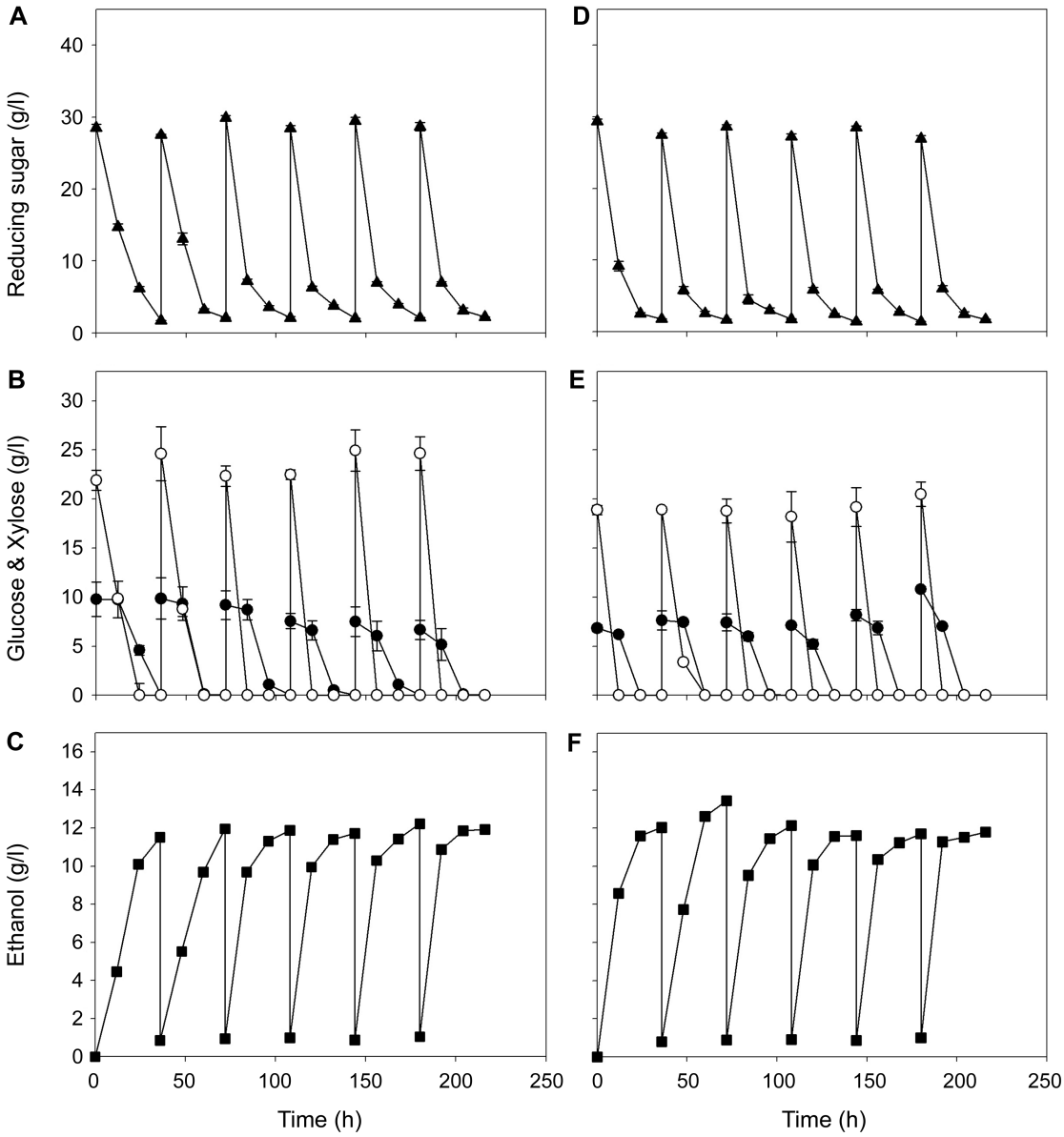

Fig. 2. Repeated-batch fermentor cultures using a surface-aerated fermentor for bioethanol production.
The medium was replaced every 36 h, and air was supplied into the headspace of the fermentor at a rate of 30 (**A**, **B**, and **C**) and 100 mL/min (**D**, **E**, and **F**). (**A**, **D**) Reducing sugar concentration in the culture supernatant. (**B**, **E**) Glucose (○) and xylose (●) concentrations in culture supernatant. (**C**, **F**) Bioethanol production. All measurements were conducted three times (n=3) using the same sample, and the average and standard deviations were calculated. The data are partially quoted from our previous work [17].

Maximum cell growth, when the medium replacement time was 36 h and 48 h, ranged from 10–12 based on the OD_{600} (optical density at 600 nm) (data not shown). In fact, the CSH medium had a turbid and dark color owing to the colors of the hydrolysate of seaweed *S. sagamianum* and CSL. Therefore, the initial cell OD_{600} of the culture broth was higher than the value of when peptone and yeast extract were used. Although cell growth was monitored in this experiment, the conventional growth curve pattern was not observed and only an increase in the OD_{600} was observed.

Although no data are shown, two other kinds of media were investigated for the preparation of a practical and cost-effect medium for bioethanol production from the hydrolysate of *S. sagamianum*. One was the medium preparations that only consisted of the hydrolysate of *S. sagamianum*, and the other medium preparation consisted of both CSL and the hydrolysate of *S. sagamianum*. However, these two media preparations were not adequate for long-term bioethanol production, because bioethanol production, cell growth, and sugar consumption gradually

decreased after the second batch of the repeated-batch operation. This occurred because CSL itself contained, to a certain extent, the nutrient ingredients needed for cell growth and bioethanol production, but was not sufficient for efficient cell growth and bioethanol production. Therefore, we decided to use the CSH medium in the repeated-batch operation for bioethanol production from the hydrolysate of *S. sagamianum*.

In our previous study, we investigated the optimal surface aeration rate in the repeated-batch fermentor culture for bioethanol production using the hydrolysate of seaweed *S. sagamianum* [17]. The optimal surface aeration rates were determined to be between 30 and 100 ml/min in a working volume of 880 ml in a 2.5 l fermentor. In this section, we investigated the performance of bioethanol production in the repeated-batch fermentor at surface aeration rates ranging from 30 to 100 ml/min in a working volume of 880 ml in a 2.5 l fermentor. A surface aeration rate of 100 ml/min corresponds to an oxygen transfer coefficient of about 0.053 (min^{-1}) [17]. This value was remarkably lower than the value obtained when the shaking flask culture was conducted. As shown in Fig. 2, surface aeration rates of 30 and 100 ml/min were used during the repeated-batch operation for 6 runs (216 h). In these experiments, before medium replacement, the maximum bioethanol concentrations were about 11–12 g/l (Fig. 2C and 2F) and all monosaccharides including glucose and xylose were completely consumed (Fig. 2B, 2E, and 3). Glucose, xylose, fructose, and mannose were previously identified as the major monosaccharides in the hydrolysate of seaweed *S. sagamianum* [31]. Glucose was completely consumed before 24 h, and xylose was completely consumed after 36 h (Fig. 3). After this time, fructose and mannose were only present in trace amounts. The maximum cell growths shown in Fig. 2A and 2D were also about 10–12 as measured by OD_{600} (data not shown).

Throughout the repeated-batch culture using CSH medium, we demonstrated that bioethanol production could be carried out repeatedly and steadily for up to 216 h, where the total cumulative bioethanol production (CEP) was 57.9 (g-ethanol) and 58.0 (g-ethanol) when the surface aeration rates were 30 ml/min and 100 ml/min, respectively (Fig. 4A and 4C). In addition, the CSC (cumulative sugar consumption) and CEP were linearly related. Using this relationship, the bioethanol yield could be calculated based on the sugar consumed (Fig. 4B and 4D). The bioethanol yields were calculated based on sugar levels using the DNS assay and quantitative TLC analysis. The CSC, which was measured using the DNS assay, and the TLC analysis produced very similar values. However, the values determined by DNS should be theoretically higher than those determined by quantitative TLC analysis, since only glucose and xylose were considered in the TLC analysis. However, as shown in Fig. 4B and 4D, this was not observed, which may have occurred if the DNS assay underestimated the CSC. The bioethanol yields, which were calculated from the slopes of Fig. 4B and 4D and were averaged with the values determined from both the DNS assay and quantitative TLC analysis, were 0.43 (about 84% of theoretical value) and 0.44 (about 86% of theoretical value) when the surface aeration rates were 30 ml/min and 100 ml/min, respectively (Fig. 4B and 4D). These two values were very close to those reported in a previous study [31], in which the bioethanol yield was determined to be approximately 0.43 based on quantitative TLC analyses of glucose and xylose. Since the bioethanol yields in this study were determined to be close to that reported in the previous report, it is highly likely that the DNS assay underestimated the CSC

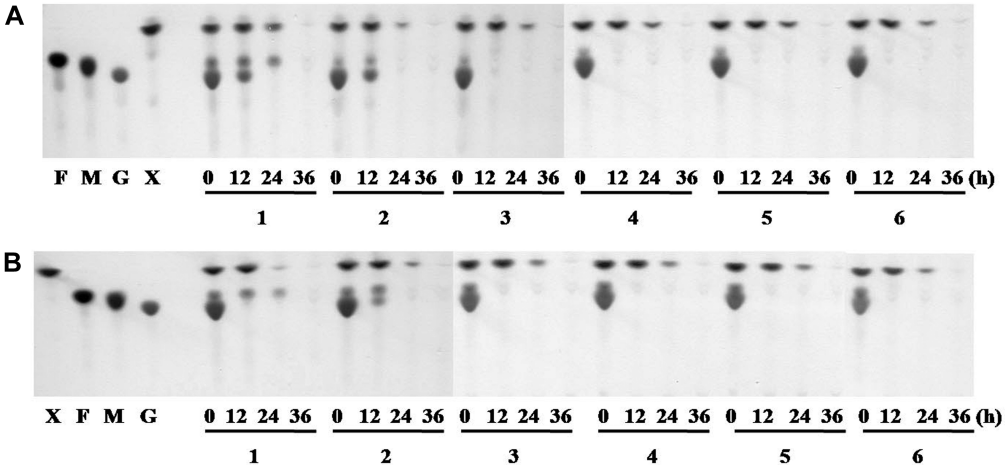

Fig. 3. TLCs of the culture supernatant of the repeated-batch fermentor culture shown in Fig. 2.
G, X, F, and M indicate glucose, xylose, fructose, and mannose standards, respectively. Air was supplied into the headspace of the fermentor at a rate of 30 (**A**) and 100 ml/min (**B**). Numbers below the elapsed times indicate the consecutive run numbers in the repeated-batch fermentor culture. The data are partially quoted from our previous work [17].

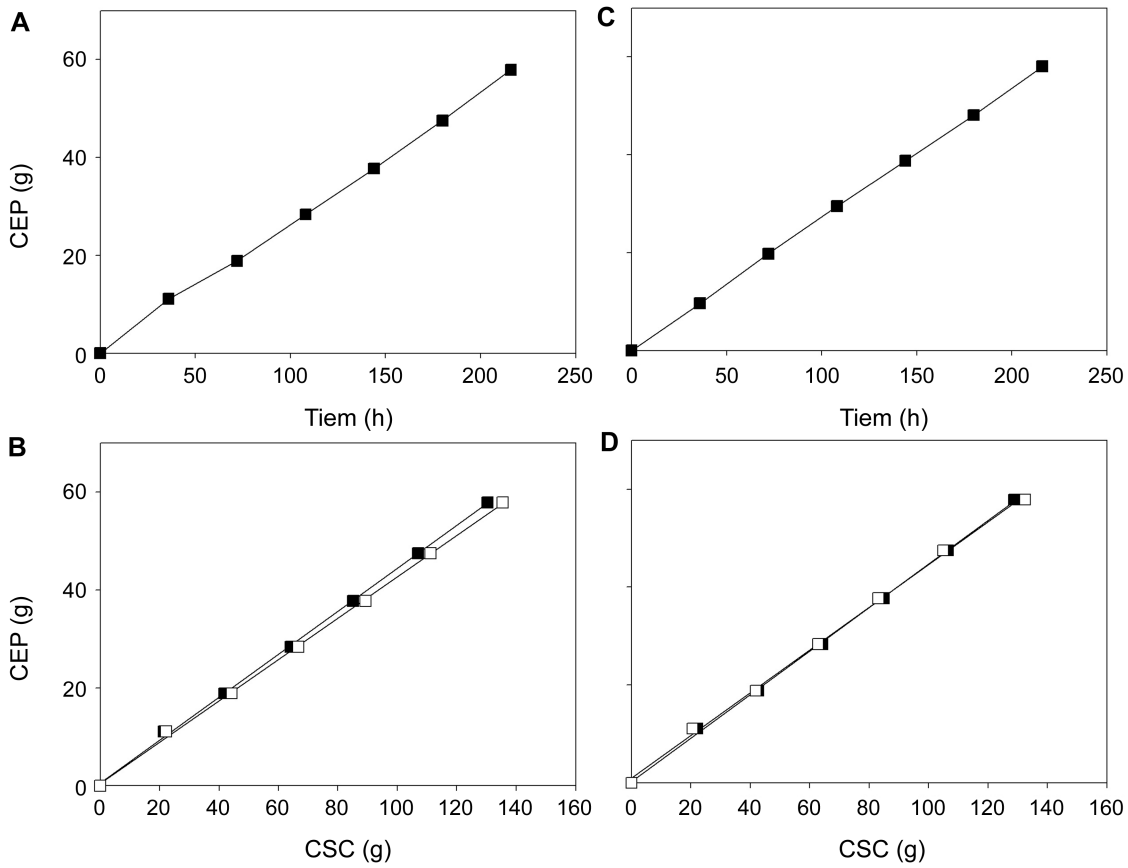

Fig. 4. Cumulative bioethanol production (CEP) from Fig. 2.
Bioethanol production and sugar consumption (by DNS assay and quantitative TLC analysis) in the withdrawn culture broths in the fermentor culture were cumulated consecutively. (**A**, **C**) Time-course of CEP. (**B**, **D**) CEP vs. CSC (cumulative sugar consumption). Air was supplied into the headspace of the fermentor at a rate of 30 (**A** and **B**) and 100 ml/min (**C** and **D**). In (**B**) and (**D**), closed (■) and open squares (□) indicate the data from the DNS assay and quantitative TLC, respectively. All the data were calculated from the average values of Fig. 2. The data are partially quoted from our previous work [17].

value. This underestimation may have occurred if the medium preparation using the hydrolysate of seaweed *S. sagamianum* and CSL interfered with the DNS assay.

Although there have only been a limited number of reports on bioethanol production from seaweed, in which the bioethanol was produced from the hydrolysates of microalgal biomass [7], *Saccharina latissima* [1], *Laminaria hyperborean* [8], and brown algae [18], seaweed has recently gained attention as a potential alternative bioenergy resource, particularly for bioethanol. In this study, the repeated-batch bioethanol production process was successfully achieved using CSL as an organic N-source. The medium used for the bioethanol production from the hydrolysate of seaweed *S. sagamianum* consisted of CSL and some salts, and these ingredients were dissolved in the hydrolysate of seaweed *S. sagamianum*. Because CSL is a by-product of corn processing, the medium preparation used in this study is a potentially practical and cost-effective method for bioethanol production. However, successful application of this medium preparation must be tested on a commercial production scale. In addition, in this study, we demonstrated that this medium preparation worked well at an optimal surface aeration rate for the bioethanol production from xylose.

Acknowledgment

This study was carried out with the support of the Korea Ocean Research and Development Institute (Project No. PP00740), Republic of Korea.

References

1. Adams, J. M., J. A. Gallagher, and I. S. Donnison. 2009. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. *J. Appl. Phycol.* **21:** 569–574.
2. Amartey, S. and T. W. Jeffries. 1994. Comparison of corn steep liquor with other nutrients in the fermentation of D-xylose by *Pichia stipitis* CBS 6054. *Biotechnol. Lett.* **16:** 211–214.
3. Chaplin, M. F. and J. F. Kennedy. 1986. *Carbohydrate Analysis; A Practical Approach,* pp. 3. IRL Press, Oxford, UK.

4. Goh, C. S. and K. T. Lee. 2010. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. *Renew. Sustain. Energy Rev.* **14:** 842–848.
5. Gouda, M. K., A. E. Swellam, and S. H. Omar. 2001. Production of PHB by a *Bacillus megaterium* strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. *Microbiol. Res.* **156:** 201–207.
6. Han, J. G., S.-H. Oh, M.-H. Jeong, S.-S. Kim, H.-B. Seo, K.-H. Jung, *et al*. 2009. Two-step high temperature pretreatment process for bioethanol production from rape stems. *KSBB J.* **24:** 489–494.
7. Harun, R., M. K. Danquah, and G. M. Forde. 2010. Microalgal biomass as a fermentation feedstock for bioethanol production. *J. Chem. Technol. Biotechnol.* **85:** 199–203.
8. Horn, S. J., I. M. Aasen, and K. Østgaard. 2000. Ethanol production from seaweed extract. *J. Ind. Microbiol. Biotechnol.* **25:** 249–254.
9. Horn, S. J., I. M. Aasen, and K. Østgaard. 2000. Production of ethanol from mannitol by *Zymobacter palmae*. *J. Ind. Microbiol. Biotechnol.* **24:** 51–57.
10. Hull, S. R., B. Y. Yang, D. Venzke, K. Kulhavy, and R. Montgomery. 1996. Composition of corn steep water during steeping *J. Agric. Food Chem.* **44:** 1857–1863.
11. John, R. P., G. S. Anisha, K. M. Nampoothiri, and A. Pandey. 2011. Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresour. Technol.* **102:** 186–193.
12. Kadam, K. L. and M. M. Newman. 1997. Development of a low-cost fermentation medium for ethanol production from biomass. *Appl. Microbiol. Biotechnol.* **47:** 625–629.
13. Kona, R. P., N. Qureshi, and J. S. Pai. 2001. Production of glucose oxidase using *Aspergillus niger* and corn steep liquor. *Bioresour. Technol.* **78:** 123–126.
14. Lawford, H. G. and J. D. Rousseau. 1997. Corn steep liquor as a cost-effective nutrition adjunct in high-performance *Zymomonas* ethanol fermentations. *Appl. Biochem. Biotechnol.* **63–65:** 287–304.
15. Lee, P. C., S. Y. Lee, S. H. Hong, and H. N. Chang. 2003. Batch and continuous cultures of *Mannheimia succiniciproducens* MBEL55E for the production of succinic acid from whey and corn steep liquor. *Bioprocess Biosyst. Eng.* **26:** 63–67.
16. Lee, P. C., W. G. Lee, S. Y. Lee, H. N. Chang, and Y. K. Chang. 2000. Fermentative production of succinic acid from glucose and corn steep liquor by *Anaerobiospirillum succiniciproducens*. *Biotechnol. Bioprocess Eng.* **5:** 379–381.
17. Lee, S.-E., H.-J. Kim, W. Y. Choi, D. H. Kang, H.-Y. Lee, and K.-H. Jung 2011. Optimal surface aeration rate for bioethanol production from the hydrolysate of seaweed *Sargassum sagamianum* using *Pichia stipitis. KSBB J*. **26:** 311–316.
18. Lee, S. M., J. H. Kim, H. Y. Cho, H. Joo, and J. H. Lee. 2009. Production of bioethanol from brown algae by physicochemical hydrolysis. *J. Korean Ind. Eng. Chem.* **20:** 517–521.
19. Maddipati, P., H. K. Atiyeh, D. D. Bellmer, and R. L. Huhnke. 2011. Ethanol production from syngas by *Clostridium* strain P11 using corn steep liquor as a nutrient replacement to yeast extract. *Bioresour. Technol.* **102:** 6494–6501.
20. Mosier, N., R. Hendrickson, N. Ho, M. Sedlak, and M. R. Ladisch. 2005. Optimization of pH controlled liquid hot water pretreatment of corn stover. *Bioresour. Technol.* **96:** 1986–1993.
21. Nobe, R., Y. Sakakibara, N. Fukuda, N. Yoshida, K. Ogawa, and M. Suiko. 2003. Purification and characterization of laminaran hydrolases from *Trichoderma viride*. *Biosci. Biotechnol. Biochem.* **67:** 1349–1357.
22. Rivas, B., A. B. Moldes, J. M. Domínguez, and J. C. Parajó. 2004. Development of culture media containing spent yeast cells of *Debaryomyces hansenii* and corn steep liquor for lactic acid production with *Lactobacillus rhamnosus*. *Int. J. Food Microbiol.* **97:** 93–98.
23. Robyt, J. F. and R. Mukerjea. 1994. Separation and quantitative determination of nanogram quantities of maltodextrins and isomaltodextrins by thin-layer chromatography. *Carbohydr. Res.* **251:** 187–202.
24. Schroeder, H. W. 1966. Effect of corn steep liquor on mycelial growth and aflatoxin production in *Aspergillus parasiticus*. *Appl. Environ. Microbiol.* **14:** 381–385.
25. Seo, H.-B., S. S. Kim, H.-Y. Lee, and K.-H. Jung. 2009. High-level production of ethanol during fed-batch ethanol fermentation with a controlled aeration rate and non-sterile glucose powder feeding of *Saccharomyces cerevisiae*. *Biotechnol. Bioprocess Eng.* **14:** 591–598.
26. Silveira, M. M., E. Wisbeck, I. Hoch, and R. Jonas. 2001. Production of glucose-fructose oxidoreductase and ethanol by *Zymomonas mobilis* ATCC 29191 in medium containing corn steep liquor as a source of vitamins. *Appl. Microbiol. Biotechnol.* **55:** 442–445.
27. Sreenath, H. K. and T. W. Jeffries. 1996. Effect of corn steep liquor on fermentation of mixed sugars by *Candida shehatae* FPL-702. *Appl. Biochem. Biotechnol.* **57–58:** 551–561.
28. Sreenath, H. K. and T. W. Jeffries. 2000. Production of ethanol from wood hydrolyzate by yeasts. *Bioresour. Technol.* **72:** 253–260.
29. Wi, S. G., H. J. Kim, S. A. Mahadevan, D.-J. Yang, and H.-J. Bae. 2009. The potential value of the seaweed Ceylon moss (*Gelidium amansii*) as an alternative bioenergy resource. *Bioresour. Technol.* **100:** 6658–6660.
30. Yeon, J.-H., S.-E. Lee, W. Y. Choi, W.-S. Choi, I.-C. Kim, H.-Y. Lee, and K.-H. Jung. 2011. Bioethanol production from the hydrolysate of rape stem in a surface-aerated fermentor. *J. Microbiol. Biotechnol.* **21:** 109–114.
31. Yeon, J.-H., S.-E. Lee, W. Y. Choi, D. H. Kang, H.-Y. Lee, and K.-H. Jung. 2011. Repeated-batch operation of surface-aerated fermentor for bioethanol production from the hydrolysate of seaweed *Sargassum sagamianum*. *J. Microbiol. Biotechnol.* **21:** 323–331.
32. Yeon, J.-H., H.-B. Seo, S.-H. Oh, W.-S. Choi, D. H. Kang, H.-Y. Lee, and K.-H. Jung. 2010. Bioethanol production from hydrolysate of seaweed *Sargassum sagamianum. KSBB J.* **25:** 283–288.
33. Yoon, J. J., Y. J., Kim, S. H. Kim, H. J. Ryu, J. Y. Choi, G. S. Kim, and M. K. Shin. 2010. Production of polysaccharides and corresponding sugars from red seaweed. *Adv. Mater. Res.* **93–94:** 463–466.