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연구논문

미세조류 *Microcystis aeruginosa*로부터 바이오 알콜의 생산

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Bioalcohol Production with Microalgae, *Microcystis aeruginosa*

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Abstract: The microalgae, *Microcystis aeruginosa* are able to proliferate in a wide range of freshwater ecosystem. *M. aeruginosa* was cultivated in 25 L and 240 L race-way reactor containing modified medium with added urea 0.2 g/L, increased Fe^{+2}, and decreased Ca^{+2}ion compared to BG11 medium. Sugar contents of *M. aeruginosa* grown in BG11 medium, and modified medium were 120 mg/mL and 140 mg/mL respectively. Fermentation was conducted with the extract of *M. aeruginosa* at 30℃ for 30 h, using *Saccharomyces cerevisiae* (Sc), *Pichia stipitis* (Ps), *Zymomonas mobilis* (Zm), and mixed-culture of these strains (Sc + Ps + Zm). *Pichia stipitis* (0.7%) was found to be more suitable for producing bioalcohol from *M. aeruginosa* extract than other strains of *Saccharomyces cerevisiae* (0.45%) and *Zymomonas mobilis* (0.61%), while mixed-cultured of these strains showed higest productivity by 1.75%. Biomass of *M. aeruginosa* contains the potency to be the most renewable resource for bioalcohol fermentation.

Keywords: Bioalcohol, *Microcystis aeruginosa*, mixed strains, urea

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1. Introduction

Rising energy consumption, depletion of fossil fuels and increased environmental concerns has shifted the focus of energy generation towards biofuel use. Global crude oil production is predicted to decline five times below its current level by 2050 [1]. Bioethanol is the major surrogate for liquid fossil fuels. However, large-scale production of bioethanol is being increasingly criticized for its use of food sources as raw material. The use of sugar rich feedstocks like sugar cane, sugar beet, corn, and wheat for bioethanol production causes the escalation of food prices. To overcome the disadvantages, "second generation" biofuels which are opposed to "first generation" biofuels, were optimized based on the bioethanol from lignocellulosic materials, such as agricultural by-products, forest residues, industrial waste steams or energy crops [2]. Lignocellulosic biomass requires pre-treatment before they can be used for biofuel (bioethanol) production. Present day's technologies for valorization of these components uses high temperature and pressure for pretreatment which yields less fermentable sugars and increases the cost of overall process. Also, by-products generated during the process have adverse affect on environment which still needs to be resolved [3].

Both "generation" biofuels have their own limitations, therefore research has focused on microalgae-based fuels. In brief, the main advantages of microalgal based biofuels are: (1) Sequestration of CO_2 during growth (CO_2 levels are now classified as "dangerously high"): (2) Can be harvested every season such as from *Chlorella*, *Dunaliella*, *Chalamydomonas*,

Scenedesmus, and *Spirulina*: (3) Higher carbohydrates and lipids content which can be utilized as renewable and biodegradable biofuels [4]. Brennan and Owende [5] has listed the desirable characteristics of algal strains to be considered as candidates for biofuel production, such as (1) robust and able to survive the shear stresses common in photobioreactors (2) able to dominate wild strains in open pond production systems; (3) high CO_2 sinking capacity; (4) limited nutrient requirements; (5) tolerant to a wide range in temperatures resulting from the diurnal cycle and seasonal variations; (6) potential to provide valuable co-products; (7) fast productivity cycle; (8) high photosynthetic efficiency, and (9) display self-flocculation characteristics.

Microalgae have the potential to produce biofuels in large enough quantities as a realistic alternative source. Despite the higher lipid, protein and biomass contents, all other factors are important as well. In this paper we will investigate the suitability of alcohol production from microalgae *Microcystis aeruginosa.*

2. Materials and Methods

2.1. *M. aeruginosa* cultivation and harvest

M. aeruginosa was inoculated into modified BG11 medium and BG11 medium in 25 L photoreactor and 240 L raceway reactor agitated by paddle wheels (45 rpm) at 25℃, for 20 days under LED light (60 $\mu Em^{-2}s^{-1}$) with 18 h light and 6 h dark photoperiod. After cultivation, *M. aeruginosa* was precipitated with the combination of 5 μM Al_2SO_4 and 1 μM $FeCl_3$, the precipitates were hydrolyzed with c-H_2SO_4, followed by autoclaving and sonication. The final extract was used as substrate for total carbohydrate analysis and alcohol fermentation.

2.2. Carbohydrate analysis

Moisture, crude protein, crude lipid, and ash content were determined using the standard methods described by the Association of Official Analytical Chemists [6]. Protein content was analyzed using the semi-Kjeldahl method [7]. Lipids were extracted with anhydrous diethyl ether using a Soxhlet apparatus. Moisture was quantified by oven-drying the samples (1-2 g) at 105℃ for 12 h. Ash was determined after incineration in a furnace at 550℃. Total carbohydrate content was calculated by subtracting the sum of moisture, crude protein, crude lipid, and ash mass from that of the total sample (100 g) [8].

100 mg dry weight of *M. aeruginosa* was mixed with 5.0 mL of 2 M HCl in a test tube and N_2 gas was supplied to replace the inner air and was capped, then hydrolysed in heating block at 100℃ for 5 h. Sample was cooled, neutralized with 2 N NaOH and centrifuged at 6,000 rpm (650 xg). Supernatant was filtrated with 0.45 μm membrane filter. The filtrate sample analyzed with HPLC (Fluorescence detector Ex = 320, Em = 430, Prominence HPLC, Shimadzu Co, Ltd. Kyoto, Japan), which was injected to Shim-pack column ISA-07 (4.0 mm × 250 mm) [9,10]. The mobile phase was changed step by step from potassium borate (pH 8) to potassium borate (pH 9). The flow rate was 0.6 mL/min and the reagent used was 1% arginine in 3% boric acid (0.5 mL). The reaction temperature was 150℃ and oven temperature was 65℃. DNS method [11] was performed for reduced sugar analysis, and dichromate method [12] was for produced alcohols.

2.3. Alcohol fermentation

Fermentation process were performed in 500 mL Erlenmeyer flasks with 200 mL extract at 30℃ for 30 h, with three types of strain: *Saccharomyces cerevisiae* (*Sc*), *Pichia stipitis* (Ps), *Zymomonas mobilis* (Zm), and mixed of these three strain (sc + ps + zm) in orbital shaking incubator (120 rpm). Samples were analysed after every 2 h.

3. Result and Discussion:

Microalgae consists the highest growth potential among the photoautotrophic organisms such as higher plants and seaweed. Bioethanol production from microalgae as a raw source has not been studied intensively. We found that ratio of carbohydrate content in *Microcystis aeruginosa* is remarkably higher than other components which indicate the potential of *Microcystis aeruginosa* as renewable source for alcohol production (Table 1). It contained wide range of fermentable sugars that can be converted into alcohols using *Saccharomyces cerevisiae* (Sc) and *Zymomonas mobilis* (Zm) for Suc, Mal, Lac, Man, Gal and Glc, and *Pichia stipitis* (Ps) for xyl [15-17] and rham [18] (Table 2). To increase the biomass of the microalgae, we used modified medium against BG11 medium. *M. aeruginosa* showed higher growth in modified medium than original BG11 medium. The peculiarity of modified medium is addition of urea, increased ingredient for P, Fe^{+2} ion, and decreased Ca^{+2} ion compared with BG11 medium (Table 3 and Fig. 1, Fig. 2). We found that Urea served as convenient source of nitrogen with higher growth and sugar contents than $NaNO_3$ for N-source. Urea (0.2 g/L) was biomass productive for *M. aeruginosa* growth (Fig. 3). Also, with the change of Fe^{+2} and Ca^{+2} concentration in the growth medium, *M. aeruginosa* showed higher growth rate (O.D. $_{Fe+2}$ = 0.8; O.D. $_{Ca+2}$ = 0.89) and glucose concentration (Fe^{+2}-100 mg/L; Ca^{+2}-150 mg/L) at 40 mg/L than 6, 60, 80 and 100 mg/L of Fe^{+2} and Ca^{+2}. In both condition, we observed that the

reducing sugar concentration was higher (Fe^{+2}-113 mg/L; Ca^{+2}-145 mg/L) on 4th day, however the growth rate was lower (O.D. $_{Fe+2}$ = 0.52; O.D. $_{Ca+2}$ = 0.58). Urea provides the marvelous nutritional requirement which supported for higher cellular mass as well as carbohydrate increment. Therefore, the seven day growing condition for biomass yield would be useful for increasing comparative reducing sugar concentration. More specifically, Fe^{+2} concentrations at lower or higher than 40 mg/L reduced the growth rate and reducing sugar concentration (Fig. 4), whereas optimum concentration of Ca^{+2} remarkably increased the growth rate (Fig. 5) than other components which shows *M. aeruginosa* is Fe^{+2} and Ca^{+2} concentration dependent.

Table 1. General composition of *M. aeruginosa*

Component	Moisture	Ash	Lipid	Protein	Carbohydrate
Contents (%)	10.58	25.69	0.35	6.96	56.42

Table 2. Carbohydrate composition of *Microcystis aeruginosa*

Sugars	Contents (μg/500 mg)	Sugars	Contents (μg/500 mg)
Sucrose	3496.85	Mannose	2.38
Cellobiose	232.05	Arabinose	20.43
Maltose	57.64	Galactose	262.64
Lactose	0.88	Xylose	343.65
Rhamnose	893.53	Glucose	1912.66
Ribose	118.37		

Table 3. Ingredient composition of BG11 and Modified medium

	BG11 medium	Modified medium
Components	Contents (g/L)	Contents (g/L)
$NaNO_3$	1.5	1.5
K_2HPO_4	0.04	0.6
Ferric ammonium citrate	0.006	0.04
$CaCl_2{\cdot}2H_2O$	0.036	0.001
$MgSO_4{\cdot}7H_2O$	0.075	0.075
Na_2CO_3	0.02	0.02
EDTA	0.001	0.001
Citric acid	0.006	0.006
Urea	0	0.2

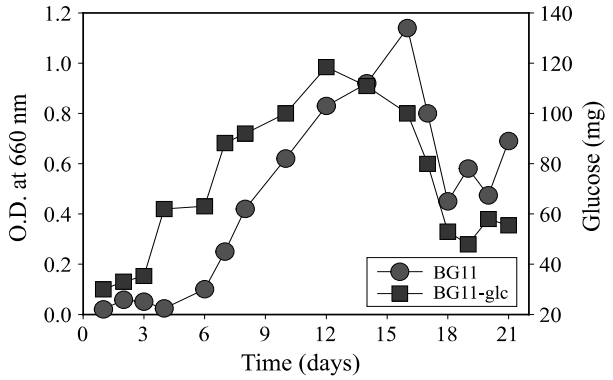

Fig. 1. Biomass and sugar contents of *M. aeruginosa* in BG11 medium.

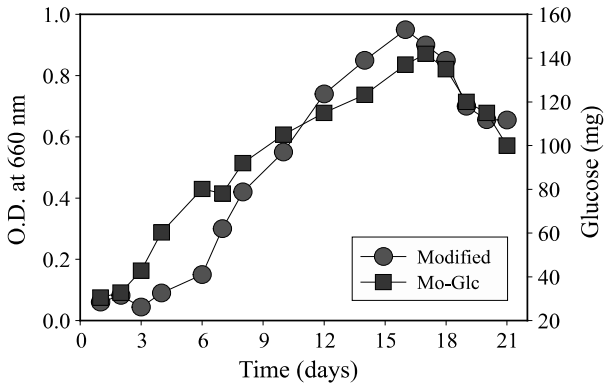

Fig. 2. Biomass and sugar contents of *M. aeruginosa* in modified BG11 medium.

The growth and reducing sugar contents of *M. aeruginosa* in BG11 medium were remarkably decreased after 15 days (Fig. 1), but in modified medium, the parameters have been significantly increasing and slowly decreased (Fig. 2) after 18 days. Sugar contents of *M. aeruginosa* grown in BG11 medium, and modified medium were 120 mg/mL and 140 mg/mL respectively. This result shows that modified medium is more useful for effective sustaining biomass and reducing sugar than BG11 medium.

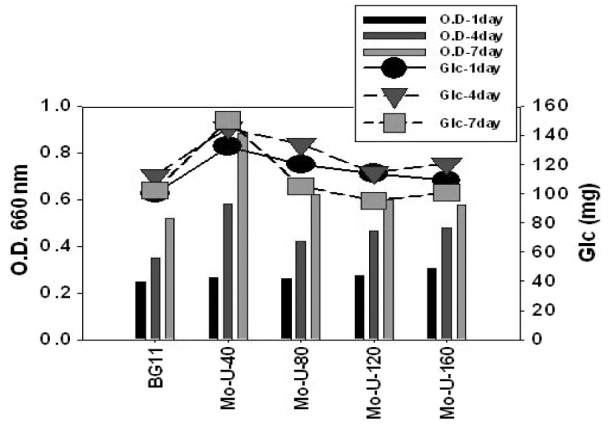

Fig. 3. Optimum urea concentration for growth and sugar content.

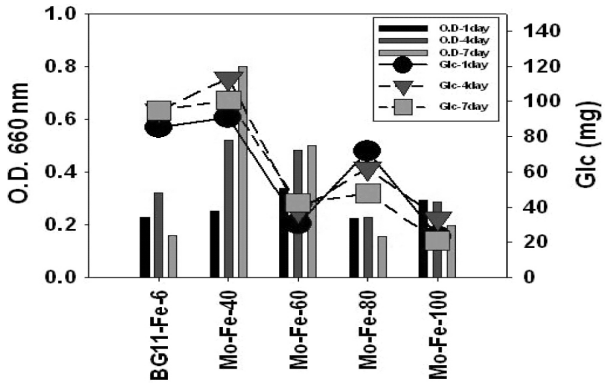

Fig. 4. Optimum Fe^{+2}concentration for *M. aeruginosa* growth and increasing sugar content.

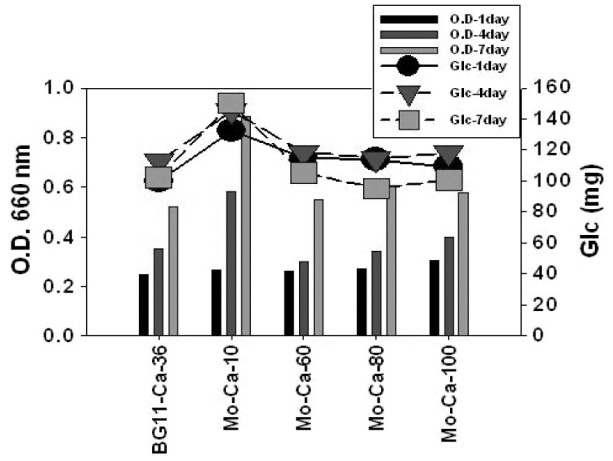

Fig. 5. Optimum Ca^{+2} concentration for *M. aeruginosa* growth and increasing sugar content.

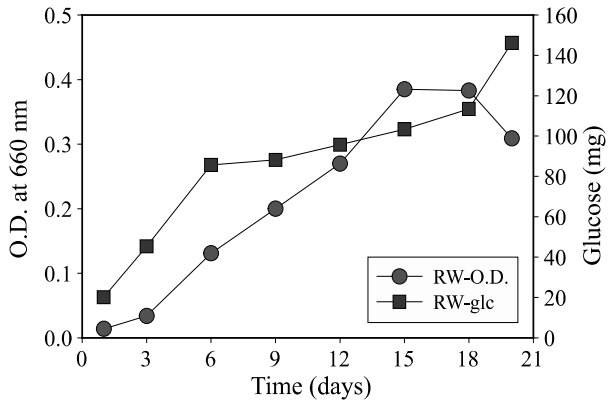

Fig. 6. Biomass and sugar contents of *M aeruginosa* in raceway reactor.

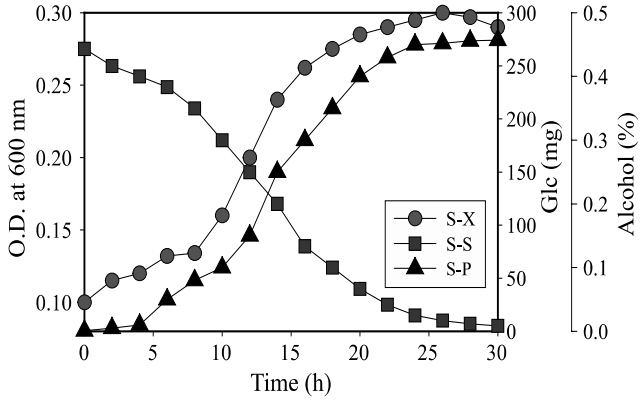

Fig. 7. Bioalcohol production with *Saccharomyces cerevisiae.*

For large scale cultivation of *M. aeruginosa*, 240 L raceway reactor was used and cultivated in modified medium under LED light (16 : 8), at 25℃ for 20 days. 140 mg/mL of sugar was obtained (Fig. 6). *M. aeruginosa* extract was fermented with 3 kinds of strain, such as *Saccharomyces cerevisiae* (Sc) [19,20] (Fig. 7), *Pichia stipitis* (Ps) [21,22] (Fig. 8), *Zymomonas mobilis* (Zm) [23] (Fig. 9) and mix-culture of these strains (Sc + Ps + Zm) (Fig. 10). Based on the result, *Pichia stipitis* was found to be more suitable for bioalcohol production (0.7%) than other strains of *Saccharomyces cerevisiae* (0.45%) and *Zymomonas mobilis* (0.61%), while mixed strains showed highest production by 1.75%. The results revealed that all the three strains are actively involved in fermentation process of their respective sugars.

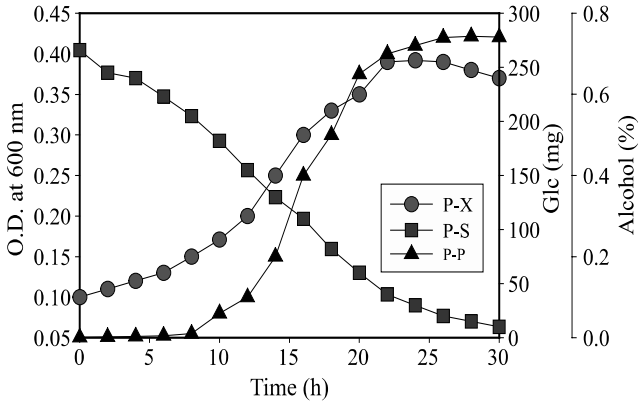

Fig. 8. Bioalcohol production with *Pichia stipiti*

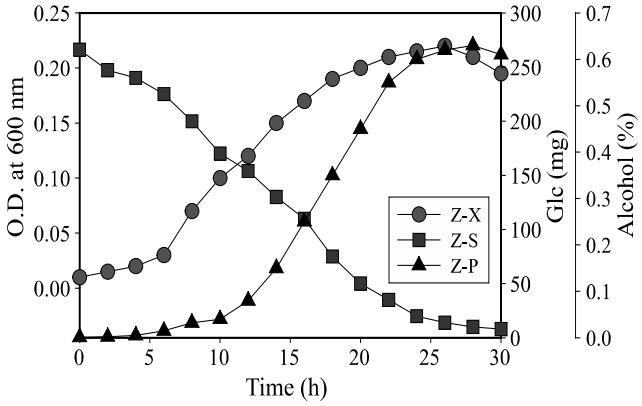

Fig. 9. Bioalcohol production with *Zymomonas mobilis.*

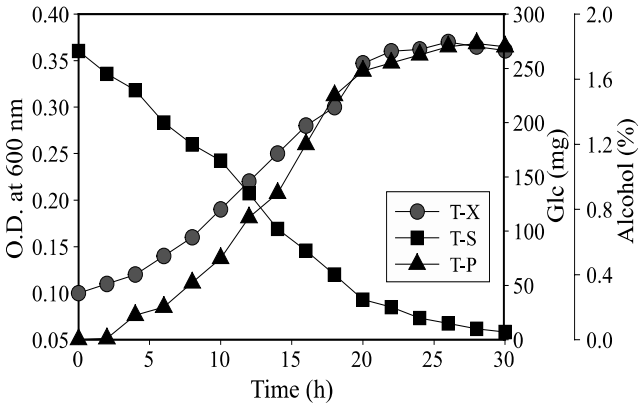

Fig. 10. Bioalcohol production with co-cultured strains (Sc + Ps + Zm).

Till the date, we could not found any such published paper by utilizing *M. aeruginosa* as raw biomass. However,

the microalgae grown under continuous light, ferments endogenously stored glycogen to equimolar amounts of acetate and ethanol when incubated anaerobically in the dark [24]. These findings along with our results reveal that *M. aeruginosa* have the potential to produce biofuels in large enough quantities as a realistic alternative source.

Agitation and temperature control are minimal requirement for batch cultivation of *M. aeruginosa*. Also, extraction of biomass was not difficult neither labor intensive. Hence, bioethanol production is expected to become an easily performed and energy-effective ethanol-production process.

With superior quality strain and higher biomass cultivation of microalgae, bioethanol production could be an effective means for CO_2 fixation and energy production. Bioethanol production process from the microalgae *M. aeruginosa* is simplified and becomes less energy intensive than lignocelluloses.

4. Conclusion

M. aeruginosa contains higher fermentable carbohydrates (56.4%), such as sucrose, cellobiose, rhamnose, galactose, xylose and glucose. Biomass of *M. aeruginosa* is easily saccharified by acid treatment followed by autoclaving. Our observation shows that these sugars obtained from *Microcystis aeruginosa* are well fermented to bioalcohol with strains: *Saccharomyces cerevisiae* (Sc), *Pichia stipitis* (Ps), Zymomonas *mobilis* (Zm) and mixed-culture of these strains (Sc + Ps + Zm). This report thus reveals the modified medium with higher growth and increased sugar content for *M. aeruginosa* cultivation as a realistic renewable source for bioalcohol production.

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