P-12 Study on the culture condition of enhancing hydrocarbon productivity of microalgaes

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ABSTRACT
In this study, we cultured fresh water microalga Botryococcus braunii the Yamanaka strain of A race with both fresh water medium and brackish water medium. It showed that brackish water medium was more efficient in recovering hydrocarbons from wet algae with n-hexane. This tendency was just the same as B race of B. braunii. Besides, microscopic observation of algae cultured at different stages showed that the high salinity medium reduced the thickness of the macromolecular substances covering the algae colony, which could explain the reason why brackish water medium was more effective in recovering hydrocarbons.

1. Introduction
It’s well known that because of the greenhouse effect and environmental pollution caused by continuously use of fossil fuel, microalgae, as an alternative energy resource, has been the limelight of the world. Especially Botryococcus braunii, which can live both in brackish water and fresh water, produces large amounts of hydrocarbons. In addition, the productivity of the hydrocarbon oils is significantly higher than other kind of biomass. These hydrocarbons are produced in algal cells and released to the extracellular matrix. Unfortunately, due to the impediment of the macromolecular substance covering the algal colony, pretreatments such as heating and drying are usually required for efficient hydrocarbon extraction with non-polar solvents.

Three races of B. braunii, which are A, B and L race, have been categorized by the hydrocarbons they produce. Botryococccenes are unbranched isoprenoid triterpenes having the formula C_{3}{H_{2n+6}}. The A race produces alkadienes and alkatrienes wherein n is an odd number 23 through 31. The B race produces botryococccenes wherein n is in the range 30 through 37, while L race makes lycopadiene of tetraterpenes. Moreover, the synthetic routes of these three races are greatly diverse. According to A. Magota et al.[1], although heating treatment was considerably efficacious in recovering hydrocarbons not only for Showa strain of B race, but also for Yamanaka strain of A race and Kawaguchi-1 of B race, but the effective temperature of every strain was rather different. Furthermore, in the study of Furuhashi et al.[2], it was reported that with the mixture of Showa strain wet algae under the long-term culture of brackish water medium and non-polar organic solvents, efficient hydrocarbons recovery was achieved, comparing with fresh water medium. While in this study, we investigated if the brackish water medium is also effective to the hydrocarbon recovery of other strains such as Yamanaka strain of A race. In order to check the influence of brackish water medium on the macromolecular substances of algal colony, and the microscopic observation was also conducted.

2. Method
In this experiment, B. braunii Yamanaka strain of A-race was used. The Yamanaka strain was cultured in four types of medium which are modified Chu-13 medium, 0.2% artificial seawater medium (0.2% ASWM), 0.4% artificial seawater medium (0.4% ASWM), 0.6% artificial seawater medium (0.6 ASWM). The nutrient salts and trace metals that were not part of artificial seawater medium were added as the same weight of Chu-13 medium. The algae was cultured on the condition that sterile air with 1.3vol% carbon dioxide was aerated into the culture flask, luminous intensity was set into about 100{\textmu}mol m^{-2} s^{-1} with a light-dark cycle of 12:12 h and the temperature was set constantly at 25°C. The algae were cultured 17 days, during which algae concentration was measured by sampling 40ml culture solution once every three days. Microscopic observation was conducted with the mixture of 50 {\textmu}L algae solution and 50 {\textmu}L ink. In the last day of the culture, hydrocarbon recovery rate was measured and the relevance between artificial seawater medium and growth rate as well as hydrocarbon recovery rate was obtained.

3. Results
Without freeze drying, the rate of hydrocarbons recovered via the layer distribution of the mixture of algae solution and hexane after 60 seconds’ vibration is shown in Figure 2. The graph shows that the amounts of hydrocarbons recovered from algae cultured with 0.6% ASWM was much more than that with modified Chu-13 medium. In conclusion, the 0.6% ASWM improved the hydrocarbons extraction easily. Moreover, even though the comparison among the recovery rate of the modified Chu-13 medium, 0.2% ASWM and 0.4% ASWM shows
no significant difference, the recovery rate presents the tendency of increasing along with the growth of the medium salinity. In summary, towards the ascent of hydrocarbon recovery rate, the brackish water medium has an effect on not just the strains of B race, but also strains of A race.

Figure 1 shows the growth rate of algae cultured with the four kinds of medium. There is no significant difference between the modified Chu-13 medium and 0.2% ASWM. On the other hand, the algae growth rate of 0.4% ASWM was hindered and 0.6% ASWM’s was much more hindered than 0.4% ASWM’s. Although the significant difference among the four mediums couldn’t be verified during the first four days of culture, in the later culture period, the growth speed significantly declined because of the high salinity. As to 0.4% ASWM, because the algae may have been adapting to the medium slowly, the growth rate of it raised in the subsequent stage.

According to Figure 3, in the fourth day of culture the macromolecular substance thickness of the 0.4% ASWM and 0.6% ASWM markedly reduced. Nevertheless, that situation had continued to the sixteenth day. In the twentieth day, the macromolecular substance of the 0.6% ASWM almost vanished, while the 0.4% ASWM’s virtually still kept the same thickness. It can be seen that the reason why the brackish water medium can ensure efficient hydrocarbons extracted with the mixture of wet algae and hexane is that the high salinity medium reduces the thickness of the macromolecular substance covering the algal colony, and in a certain range, the higher salinity, the thinner the substance will be.

4. Summary
The same as B race, brackish water medium was also effective in recovering hydrocarbons from Yamanaka strain of A race. However, we also confirmed that brackish water medium had a bad effect on algal growth. With the microscopic observation, the reason that the brackish water medium culture could ensure efficient hydrocarbons extracted with the mixture of wet algae and hexane has been shown more intuitively. The graphs of observation showed that the culture in high salinity medium reduced the thickness of the macromolecular substance covering the algal colony.

【References】

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