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Variation of Lip Content in Microalgae, *Chlorella sp*.

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Abstract: The lipid accumulation of *Chlorella* sp. was studied based onpH of culture medium and nutrient limitation in terms of inorganic nitrogen input were also investigated using BG-11 as a basal medium. The total lipid contents(%) evaluated byvarying initial pH at 6, 7, 8 and 9 of the culture medium were 2.36 ± 0.65, 3.46 ± 0.36,4.44 ± 0.21 and 3.86 ± 0.82respectively. Moreover, the effect of inorganic nitrogen on lipid content was investigated by using $NaNO_3$ (90000 mg/L, 60000mg/L and 30000 mg/L)as nitrogen source in culture media . The highest lipid content was found that of applying 30000 mg/L $NaNO_3$ in culture medium was (3.87 ± 0.35) percent.

Keywords: *Chlorella*sp, pH, $NaNO_3$, Lipid content

1. Introduction

Biodiesel production from microalgae is an emerging technology considered by many as a very promising source of energy, mainly because of its reduced competition for land. Among these, especially, microalgae were found to be an alternative nature source of renewable petroleum resources thatis capable of meeting the global demand for fuels [1]. According to the reference [2], the idea of using algae as a source of fuel is not new, but it is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warm that is associated with burning fossil fuels. It is reported that microalgae can provide several different types of renewable biofuels which include, methane, biodiesel and biohydrogen [3]. Microalgae are faster in growth in the marine environment and yield of oil from algae is estimated between 5000 to 20000 m^3/ 4046 m^2/yr which is 7 to 31 times greater than the terrestrial crop, palm oil (635 m^3) [4]. The high growth rate of microalgae makes it possible to satisfy the massive demand on biofuels using limited land resources. Microalgae cultivation consumes less water than land crops. Most microalgae biomass contains three main components such as 1) lipids, 2) proteins, and 3) carbohydrates and/or hydrocarbons.Microalgae produce and store lipids in the form of fatty acids, phospholipids, glycolipids and it can be used as feed stocks for biodiesel production by transesterification reaction in the presence of acid or base with methanol [5]. In this research, the microalgal oil extraction for the purpose of biodiesel production from the microalgae (Chlorella sp.) was studied.

2. Materials and Methods

2.1 Raw Material

The microalgae (*Chlorella* sp.) was kindly provided by Department of Biotechnology, Technological University (Kyaukse), Kyaukse Township, Mandalay, Myanmar.

2.2 Selection of Optimum Media

The stock culture was taken from 20 ml test tubes and cultured with four different media in 500 ml glass bottles at 23˚C for selection the best growth medium of microalgae. In the culture bottles, the distilled water and each nutrient medium were 200 ml and 100 ml respectively and microalgae stock 10 ml was added. The growth rate of microalgae was counted by Haemacytometer for 9 days period. The tested four different media were BG11, MN, M4N, and DM medium (pH8, Temperature : 27-30˚C).

2.3 pH Variation

The effect of pH on the growth of *Chlorella* sp. was investigated. Four ranges of pH, namely, pH 6, pH 7, pH 8 and pH 9 were carried out to cultures. In this experiment, BG11 media was used at the temperature of 27-30˚C.

2.4 Inorganic Nitrogen Variation

$NaNO_3$ (90000 mg/L, 60000mg/L and 30000 mg/L were used as nitrogen source in BG 11 media.

2.5 Harvestingand Drying

The algae were harvested by using filter pape at 7th day of culture. After harvesting, the algae were dried at room temperature 28˚C. The dry biomass was collected, weighted and stored at room temperature for extracting microalgal oil.

2.6 Extraction of Algal Oil

The dried algae were extracted by using hexane solvent method. This method can be harmful and explosive and extracts 95% of oil. After algal oil extraction, the oil and solvent are separated by using rotary evaporator.

3. Results and Discussion

3.1 Selection of Optimum Media

The culture condition for obtaining the best growth rate of *Chlorella sp.* was tested in different nutrient media, pH values, and temperature ranges. *Chlorella sp.* was cultured with four different nutrient media (BG11, MN, M4N and DM medium) at room temperature for 9 days period. The resulted data of microalgal cells counted by haemacytometer were showed in Figure 1. It has been found that BG11 medium supported the best growth density of microalgae at 6 days.

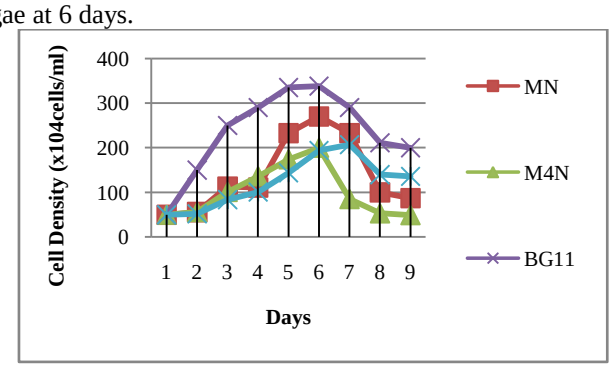

Fig. 1 Growth Density of *Chlorella sp.* Culture with Four Different Media

.3.2 pH Variation

The algae cells of *Chlorella* sp. from pH of algae were harvested to measure the lipid content. After extraction of algal oil, the oil content variations were investigated. In this study, pH variations can effect on the oil content of the algae. The results obtained from this experiment were described in Table.

3.3 Nutrient Limitation

The effect of three different nitrogen contents $NaNO_3$(90000 mg/L, 60000mg/L and 30000 mg/L) as nitrogen source in culture media on the oil content of Chlorella sp. was also examined. The results obtained from this experiment were described in Table. In nitrogen limited situations, the biomass of this algae decrease significantly and algae lipid content usually increases as a mechanism of survival, which makes cells stop its divisions and start to store energy in the form of lipids. For the economy and cost effectiveness of the culture, it was suggested that to use (30000 mg/L) of $NaNO_3$concentration on biomass and oil yield.

Table.Lipid content variation of *Chlorella* sp. on a dry matter (%) for pH variation and nutrient limitation

pH Variation	
pH	Lipid Content (%) Mean ± SD
6	2.36 ± 0.65
7	3.46 ± 0.36
8	4.44 ± 0.21
9	3.86 ± 0.82
Nutrient Limitation	
$NaNO_3$(mg/L)	Lipid Content (%) Mean ± SD
30000	3.87± 0.35
60000	2.17 ± 0.29
90000	1.83± 0.76

Conclusion

The lipid content of the algae increases when put into nutrient limiting conditions.. According to the results of the experiments, *Chlorella* sp.could be cultured with optimum pH 8 at the temperature of 27-30˚C supplemented with 3000 mg/L $NaNO_3$ for high biomass and high oil content. The microalgae, *Chlorella* sp. is a good candidate for biodiesel due to its high lipid content in addition to its easy growth.

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