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Total Lipid of Mutant *Synechococcus* sp. PCC 7002

Azlin S Azmi, Mus'ab Zainal, Sarina Sulaiman, Azura Amid, Zaki Zainudin

***Abstract*—**Microalgae lipid is a promising feedstock for biodiesel production. The objective of this work was to study growth factors affecting marine mutant *Synechococcus* sp. (PCC 7002) for high lipid production. Four growth factors were investigated; nitrogen-phosporus-potassium (NPK) concentration, light intensity, temperature and $NaNO_3$ concentration on mutant strain growth and lipid production were studied. Design Expert v8.0 was used to design the experimental and analyze the data. The experimental design selected was Min-Run Res IV which consists of 12 runs and the response surfaces measured were specific growth rate and lipid concentration. The extraction of lipid was conducted by chloroform/methanol solvents system. Based on the study, mutant *Synechococcus* sp. PCC 7002 gave the highest specific growth rate of 0.0014 h^{-1} at 0% NPK, 2500 lux, 40^{o}C and 0% $NaNO_3$. On the other hand, the highest lipid concentration was obtained at 0% NPK, 3500 lux, 30^{o}C and 1% $NaNO_3$.

***Keywords*—**Cyanobacteria, lipid, mutant, marine *Synechococcus* sp. PCC 7002, specific growth rate.

I. Introduction

MICROALGAE are able to produce lipid in a short time. They are the oldest and simplest photosynthetic inhabit a wide range of aquatic or damp environments including arctic, freshwater, hypersaline, deep-sea (euphotic), coastal, and soda lake environments. The composition of lipid in algae can reach to about 30-75% of their dry basis. Statistics showed that the yield of oil algae was 200 times higher than the yield from vegetable or plant oils [1].

Many strategies and solutions have been developed maintaining renewable energy or fuel supply. One of solutions is by producing biodiesel using microalgal technology. There are many advantages of using microalgae as source of biodiesel production including higher photosynthetic efficiency, higher lipid production and higher growth rate compared to conventional crops oil. In addition, the properties of biodiesel from algae are similar to our current diesel, and more likely to have higher stability based on their flash point values [2].

Synechococcus is prokaryotic blue green algae (cyanobacteria) which are one of the largest genera in cyanobacteria. Cyanobacteria possess certain properties which have entitled them to be one of the most promising feedstock for bioenergy generation. The lipids are mainly present in the thylakoid membranes. Furthermore, cyanobacteria grow easily with basic nutritional requirements; they are able to survive if supplied with air [N_2 (nitrogen-fixing strains) and CO_2], water and mineral salts (especially phosphorous-containing salts) with light as the only energy source. Lastly, cultivation of cyanobacteria was therefore relatively simple and inexpensive [3].

The lipid content and quality of microalgae is highly variable within a species, as well as between different species. It can be affected by a number of environmental or culturing variables, such as growth phase, light intensity, temperature, salinity, carbon dioxide and nutrients. *Synechococcus* as source of biodiesel production can be produced by extracting oil or lipid from the cells. The yield of lipid or oil depends on many parameters. In order to obtain high yield of lipid the growth factors must be optimum. Thus, the main objective of this study was to study growth factors affecting marine mutant *Synechococcus* sp. PCC 7002 for high lipid production.

II. Materials and Methods

A. Microorganism and Media Preparation

Synechococcus sp. PCC 7002 marine strain was obtained from American Type Culture Collection (ATCC). Mutant strain of *Synechococcus* sp. PCC 7002 was previously prepared at IIUM. The strain was grown in 616 medium BG-11.

B. Shake Flask Cultivation

Marine mutant *Synechococcus* sp. PCC 7002 was cultured in 100 ml of shake flask supplied with 15% CO_2 for 30 minutes every day and last for 7 days. An inoculum size of 5% was added into the media for cultivation. All experiments were conducted in the water bath with different growth factors. The parameters were temperature (30 and 40^{o}C), light intensity (2500 and 3500 lux), $NaNO_3$ concentration (0 and 1%) and NPK concentration (0 and 0.05%). Total of 12 runs (Table I) were designed using *Min-Run Res IV* (Design Expert v8.0 software) and the response surfaces measured were specific growth rate and lipid concentration.

A. S. Azmi is with the Department of Biotechnology Engineering, Kulliyah of Engineering, International Islamic University Malaysia, P.O. Box 10, 50728 Kuala Lumpur, Malaysia (phone: +603-6196-6547; fax: +603-6196-4442; e-mail: azlinsu76@iium.edu.my).

M. Zainal was Department of Biotechnology Engineering, Kulliyah of Engineering, International Islamic University Malaysia, P.O. Box 10, 50728 Kuala Lumpur, Malaysia. (e-mail: musabzainal5@gmail.com).

S. Sulaiman, A. Amid, and Zaki Zainudin are with the Department of Biotechnology Engineering, Kulliyah of Engineering, International Islamic University Malaysia, P.O. Box 10, 50728 Kuala Lumpur, Malaysia (e-mail: sarina@iium.edu.my; azuraamid@iium.edu.my; zakizainudin@iium.edu.my).

TABLE I
DESIGN OF EXPERIMENT

Run	A:NPK % (v/v)	B : Light Intensity (Lux)	C:Temperature (°C)	D :$NaNO_3$ %(w/v)
1	0.00	2500	40.00	1.00
2	0.00	2500	30.00	1.00
3	0.05	2500	40.00	0.00
4	0.05	3500	30.00	1.00
5	0.05	2500	40.00	1.00
6	0.00	3500	30.00	1.00
7	0.05	3500	40.00	0.00
8	0.05	2500	30.00	0.00
9	0.00	3500	40.00	1.00
10	0.05	3500	30.00	0.00
11	0.00	3500	30.00	0.00
12	0.00	2500	40.00	0.00

C. Sampling and Analysis

The sample were collected and analyzed every 24 hours for 7 days. Data analysis conducted were total cell number (TCN) to determine maximum specific growth rate and lipid concentration. Lipid was extracted using Blight and Dyer [4] method. Total lipid was measured using gravimetric method.

III. RESULT AND DISCUSSION

The influence of NPK concentration, light intensity, temperature and $NaNO_3$ concentration affecting the mutant growth and lipid production were studied. Mutant *Synechococcus* sp. PCC 7002 was grown under different NPK concentrations (0% and 1% v/v), light intensities (2500 and 3500 lux), temperature (30°C and 40°C), and $NaNO_3$ concentrations (0% and 1% v/v).

Fig. 1 showed comparison of maximum specific growth rates (μ_{max}) of exponential growth obtained from 12 experimental runs (Table I). Run 12 gave the highest rate of growth which was $0.0014h^{-1}$. On the other hand, the lowest rate of growth was $0.0001h^{-1}$ obtained from run 4. The conditions of run 12 which gave highest specific growth rate were at light intensity of 2500 lux and temperature of 40°C while $NaNO_3$ and NPK concentration were both at 0% (w/v). Meanwhile, for the lowest specific growth rate of the mutant when it was cultivated under conditions of 0.05% NPK, 2500 lux, 30°C and 0% NPK.

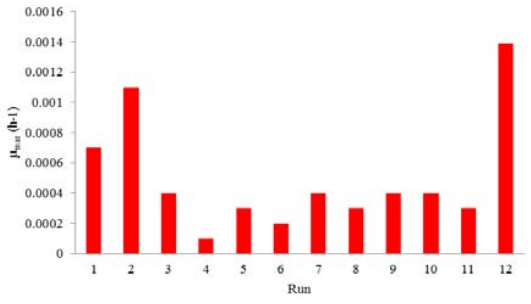

Fig. 1 Maximum of specific growth (μ_{max}) rate for mutant *Synechococcus* sp.

Figs. 2 to 4 illustrated two-factor interaction of the investigated parameters. Interesting to note that Fig. 2 showed that the highest specific growth was obtained at highest studied temperature of 40°C without $NaNO_3$ whereas many reported [5], [6] that $NaNO_3$ is a good source of nitrogen for growth. However, the specific growth rate was also increased at the other end, which at lower temperature of 30°C with 1% $NaNO_3$. Similar patent (Fig. 3) was also observed from NPK interacted with temperature where highest was obtained at 40°C without NPK. Factor interaction between $NaNO_3$ and light intensity gave highest specific growth rate at lower light intensity at 2500 lux. Interestingly the growth was highest at 40°C. Mackey et al. [7] reported that maximum growth rate temperature for marine *Synechococcus* sp. PCC 7002 is below 27°C. However, in this current study the strain was mutated, the cells might have adapted different temperature range for maximum growth.

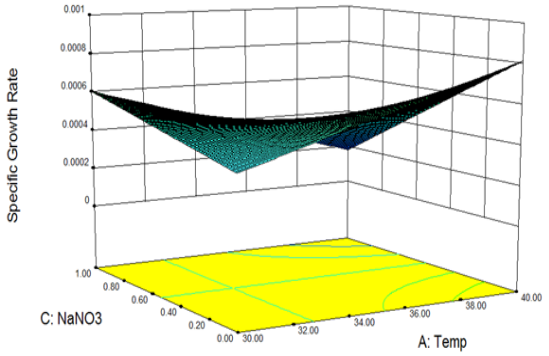

Fig. 2 Effect of temperature and $NaNO_3$ on specific growth rate of mutant *Synechococcus* sp. (PCC 7002)

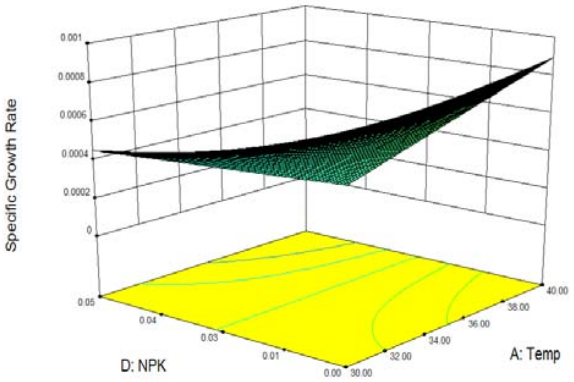

Fig. 3 Effect of NPK concentration and temperature on specific growth rate of mutant *Synechococcus* sp. (PCC 7002)

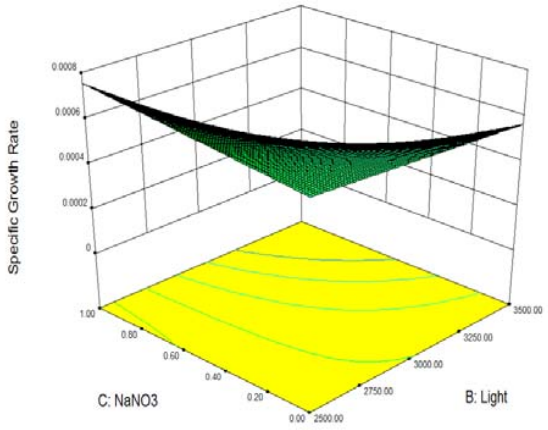

Fig. 4 Effect of $NaNO_3$ and light intensity on specific growth rate of mutant *Synechococcus* sp. (PCC 7002)

Lipid concentration extracted from mutant *Synechococcus* sp. PCC 7002 harvested from 12 experimental runs are shown in Fig. 5. The highest lipid concentration was obtained from run 6 at 0.089g/ml. The growth condition was at 0 % NPK, 3500 lux, 30°C and 1 % $NaNO_3$. The lowest concentration was from run 3 with lipid concentration at 0.003 g/ml. Run 3 growth condition was at 0.05% NPK, 2500 Lux, 40°C and 0% $NaNO_3$.

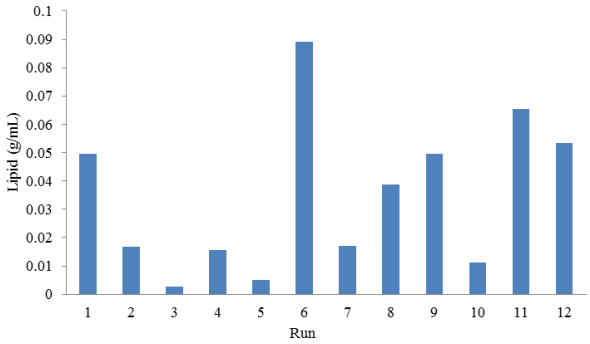

Fig. 5 Lipid concentration for 12 runs

Yield of total lipid can be affected by many parameters or factors. Figs. 6 to 9 suggested that the highest lipid was obtained when no nitrogen source was supplied (i.e. 0%) at lower end of light intensity (i.e. 2500 lux) and temperature (i.e. 30°C). This is in accordance to several reported research where nitrogen deprivation is required for lipid production. This shows that mutant *Synechococcus* sp. needs nitrogen at certain amount for initial growth and produce lipid at absent nitrogen.

The effect of temperature towards lipid production has been reported to influence variedly at certain range of temperature and for different types of microalgae. For example, study done by Converti et al. [8] showed that the lipid content of microalgae was strongly influenced by the variation of tested parameters. In their study an increase in temperature from 20 to 25°C practically doubled the lipid content of *N. oculata* (from 7.90 to 14.92%), while an increase from 25 to 30°C brought about a decrease of lipid content of *C. vulgaris* from 14.71 to 5.90%.

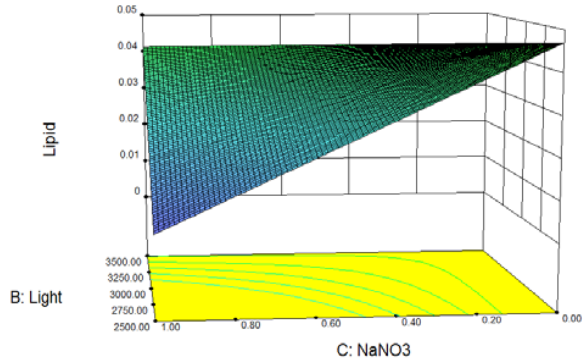

Fig. 6 Effect of light intensity and $NaNO_3$ on total lipid of mutant *Synechococcus* sp. (PCC 7002)

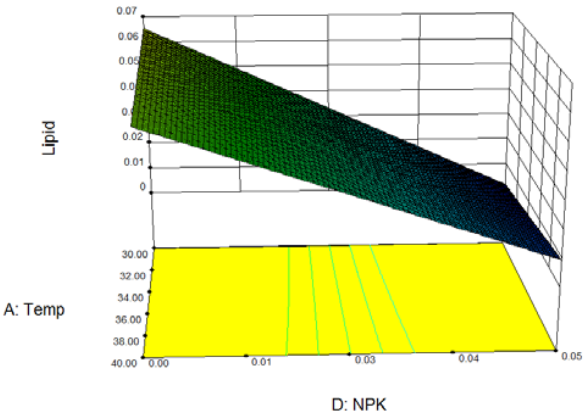

Fig. 7 Effect of NPK and temperature on total lipid of mutant *Synechococcus sp* (PCC 7002)

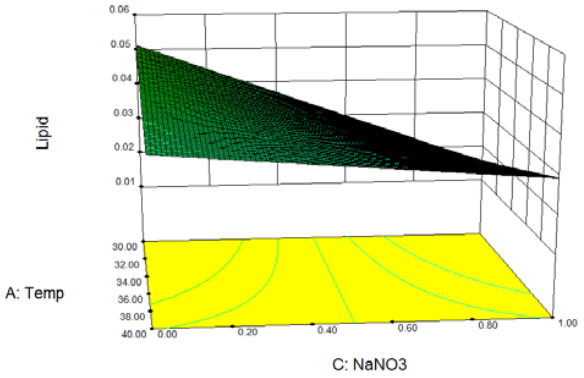

Fig. 8 Effect of $NaNO_3$ concentration and light on total lipid of mutant *Synechococcus sp* (PCC 7002)

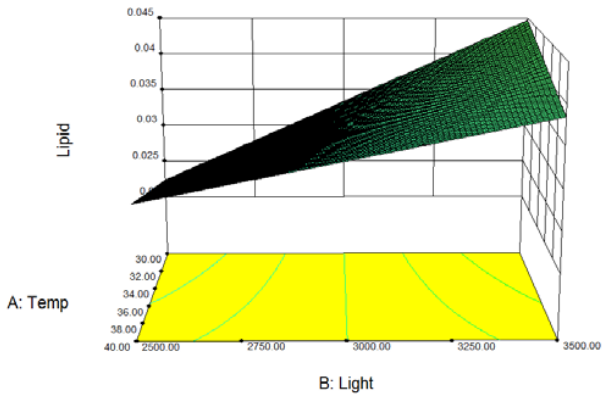

Fig. 9 Effect of temperature and light on total lipid of mutant *Synechococcus* sp. (PCC 7002)

IV. Conclusion

In order to make lipid from cyanobacteria such as *Synechococcus* sp. PCC 7002 commercially viable fuel source, research is required to ensure that the species not only able to produce as many lipids as possible, but also that it can grow at it fastest rate. Different microalgae species may have different optimum growth conditions. The variations of four parameters tested have been proved in this study to strongly influence the specific growth rate and lipid production of mutant marine *Synechococcus* sp. PCC 7002. Almost all the growth factors investigated led not only to the microalgae growth, but also to the accumulation of lipids, thereby affecting the lipids productivity. Finally, the study confirmed that nitrogen deprivation increases the lipid concentration. While other studies show highest growth requires nitrogen source, this study suggested highest specific growth rate obtained without nitrogen source.

Acknowledgment

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