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POTENTIAL OF *NOSTOC MUSCORUM* CULTURED IN BG-II MEDIUM AS BIODIESEL FEEDSTOCK SOURCE: EVALUATION OF NUTRIENT REQUIREMENT FOR CULTURE AND ITS DAILY LIPID CONTENT

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ABSTRACT

Increment of industrial development and energy demands for transportation and electricity have increased diesel-fuel uses to fulfil global energy needs. Carbon emission as impact of high fossil diesel use which pollutes the air gradually increases green house gases (GHG) and increases the intensity of acid rains. Furthermore, scarcity of fossil-fuels resources has caused high price of diesel-fuel which in turn to have increased the prices of all commodities. *Nostoc muscorum* is filamentous Cyanobacteria species which lives both terrestrial and freshwater aquatic environment. This strain has good ability in producing high biomass and potential in producing lipid. In where, *Nostoc muscorum* has potential as biodiesel feedstock alternative of food-plants sources. This study was conducting to evaluate the potential of *Nostoc muscorum* cultured in BG-II medium as biodiesel feedstock source. Evaluation of the nutrient requirement of *Nostoc muscorum* cultured in BG-II medium was done through assimilation of nitrate ($NaNO_3$)-phosphate (K_2HPO_4). Biomass production as growth parameter was measured by weighing the dried biomass for 14 days of culture. Daily lipid production was evaluated by lipid extraction using Soxhlet method. The result showed that *Nostoc muscorum* cultured in BG-II medium required 644.6795 mg/L of NO_3^- and 25.1566 mg/L of HPO_4^- with the highest biomass production 0.21 grams/300 mL. Furthermore, *Nostoc muscorum* as multicellular Cyanobacteria could grow well in BG-II medium at SGR 0.0964 µ/day. Lipid production of *Nostoc muscorum* during cultivation in BG-II for 14 days decreased day by day. The highest lipid production was reached up in day 4th of culture that was 9.53 mg/g. Based on this study, *Nostoc muscorum* has good potential as biodiesel feedstock through producing high biomass in BG-II medium.

Keywords: *Nostoc muscorum*, *Synechococcus elongatus*, Tofu wastewater, Lipid content, Cell disruption, Biodiesel.

INTRODUCTION

Limitation of non-renewable fuel resources is one of the big issues faced by the world. Whereas, the increment of global energy demands for industrial development, transportation, and electricity continuously increases diesel-fuel use. According to Energy Information Administration (EIA) of USA (2012), the usage of world liquid fuels increased by an estimation of 0.8 million bbl/d in 2011. EIA predicted that liquid fuels demand would increase by 20% (up to 1.0 million bbl/d) from 2012 to 2013. Increasing fossil-fuel use also has led some adverse impacts to environment, natural resources, economic and social aspect. Increment of carbon emission pollutes air, which gradually leads to increase greenhouse gases (GHG) in the atmosphere and the intensity of acid rains. Scarcity of fossil-fuels resources has caused high price of diesel-fuel which in turn to has increased the prices of all commodities.

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This increased diesel-fuel demand has encouraged to discovery the unconventional biodiesel sources, such as from plants, from microalgae, and from animal fats for providing a sustainable alternative energy source. Biodiesel is expected to be an important source of energy for its capability to minimize GHG emissions to atmosphere by recycling carbon and its effect on conserving the sources of fossil fuel. Biodiesel is one of the most common biofuels containing monoalkyl esters derived from organic oils, animal or plant and involves the transesterification process to convert to biodiesel (Demirbas 2007). There are four main categories of biodiesel feedstock, namely: 1) edible plant oil (soybean, rapeseed, sunflower, peanut, coconut oil, and palm), 2) non-eatable plant oil (karanja, jatropha, halophytes, sea mango, and algae), 3) waste or recycle oil, 4) oil of animals (tallow, chicken fat, yellow grease, and fish oil by-products) (Atabani *et al*. 2012).

Indonesia, as one of the main producer and exporter of palm oil, has developed biodiesel production using palm oil wastes and *Jatropha carcass*. Indonesia produced 520,000 tonnes (590 million L) of biodiesel in 2007 and it produced 2.41 billion L of biodiesel by 2010 (Zhou And Thomson 2009 *cited in* Jayed *et al*. 2011).

However, use of palm oil waste as feedstock of biodiesel still leads some environmental issues. Jayed *et al*. (2011) claimed that expansion of palm oil crop as feedstock of biodiesel becomes a threat to biodiversity caused its serious impact to environment in Southeast Asian rain forests. Many businessmen convert rainforest to palm plantation by logging and setting fire in forests. Then, palm manufacturers cause the pollution of water harming the aquatic biodiversity, such as palm oil mill effluent (POME), fertilizers, insecticides, rodenticides, and herbicides. Another impact is extinction of some animals and plants.

Non-food Cyanobacteria and microalgae have potential for biodiesel feedstocks alternative of food-plants sources. Parmar *et al*. (2011) revealed that microalgae and Cyanobacteria can produce a diverse array chemical intermediates and hydrocarbons, precursors to biofuel. Their photosynthetic system can distinguish the electrons appearing from the main processes, immediately into H_2. Its calvin cycle conducts to carbohydrates, proteins, lipids, and fatty acids production in which lipids can be derived tobe biodiesel. Singh and Gu (2010) explained some advantages of microalgae as biodiesel feedstock in their report. Microalgae can be perennial production so the amount of oil production surpasses the oil production of best crops. Otherwise, microalgae have rapid growth potency and numerous species have 20-50% biomass dry weight of oil content. In addition, the microalgae require less water than terrestrial crops thus the need of freshwater amount can be decreased. Microalgae also can support in bio-fixation of waste CO_2 and herbicides or pesticides application are not needed in their cultivation. Finally, microalgae have ability to held photobiological production of biohydrogen and also produce valuable coproducts.

Evaluation of the potential of prokaryotic microalgae, Cyanobacteria, as biodiesel feedstock becomes attractive to be studied due to their ability to produce fatty acids through photosynthesis. Cyanobacteria are oxygenic photosynthetic bacteria and also known as blue

green algae in which they take parts in global biological carbon isolation, oxygen establishment, and nitrogen cycle (Parmar *et al*. 2011). Some genera of Cyanobacteria produce poison such as freshwater *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. Oscillatoria) *rubescens*, *Synechococcus* spp., *Planktothrix* (syn. Oscillatoria) *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., some *Oscillatoria* spp.,*Schizothrix* spp. and *Synechocystis* spp. and Phormidium spp.

Nostoc muscorum is filamentous Cyanobacteria species which lives both terrestrial and freshwater aquatic environment. This strain has good ability in producing high biomass as was described by the report of Malakar *et al*. (2012), *Nostoc muscorum* was the better strain in terms of biomass, total chlorophyll content, and total N content than *Cylindrospermum majus*. Potential of lipid source is also found in this species in which it had total lipid 16.80±3.62% and its biodiesel content was 12.52±1.74% in wastewater culture (Shalaby, 2011). This study was conducted on Cyanobacteria species, namely *Nostoc muscorum* in context of determination of nutrient requirement for culture and evaluation its daily lipid content.

MATERIALS AND METHODS

The species of Cyanobacteria in this study was *Nostoc muscorum* TISTR 8164 that was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand.

Another materials included chemicals and apparatus were also used in this study. Some chemicals that were used during conducting the experiment are BG-II media consisted of $NaNO_3$, $K_2HPO_4.H_2O$, $MgSO_4.H_2O$, $CaCl_2.H_2O$, Na_2CO_3, citric acid, $Fe_2SO_4.H_2O$, EDTA, and trace elements. Water quality analysis involved chemicals such as NaOCl, HCl, $MnSO_4.H_2O$, NaOH, phenol, NH_4Cl, sulphanilamide, N-(1-naphthyl)-ethylenediamine-dihydrochloride, $NaNO_2$, copper sulphate, disodiumtetraborate, EDTA, KNO_3, H_2SO_4, potassium antimonyl tartrate, ammonium molybdate, ascorbic acid, KH_2PO_4, phenolphthalein indicator, Na_2CO_3. Then lipid extraction used petroleum ether.

The apparatus used in this study were: erlenmeyer flask, cylinder flasks 10 L, aerator, pipe, fluorescent lamp, microscope, laminar box, micropipette, tip, autoclave, volumetric pipette, vacuum filter, Whatman filter paper no.1; no.42; and GF/C, spectrophotometer, oven, centrifuge, funnel, soxhtec, muffle furnace, balance, desicator, pH meter , etc.

This research was conducted in two main steps, namely determination of nitrogen-phosphate level of species cultured in BG-II medium and evaluation of daily lipid content of species. Providing stock culture was also done in BG-II medium before starting the experiment.

1. Stock culture

Biomass cultivation was begun by preparing stock culture of Cyanobacteria species. The BG-II medium was used as standard medium for cultivating the strain as stock culture. The starter of Cyanobacteria at 5 mL was transferred aseptically into a 125 mL erlenmeyer flask containing 100 mL BG-II medium by using a disposable sterile pipette. All the strains of cultured Cyanobacteria were maintained under photoautotrophic growth condition with

continous illumination at 2000 lux. Then, the stock cultures were incubated at 28 °C with placement of stock culture on a continuous aeration. The Cyanobacteria culture might take 3 to 5 days to reach the exponential growth phase. Cultivation in BG-II was continued to 500 mL, 1000 mL, 2000 mL, and 8000 mL.

The culture of *Nostoc muscorum* was renewed once in two weeks (14 days) to ensure a regular supply of exponentially growing algal cells. Renewal of the stock culture was achieved by aseptically adding BG-II medium into the culture in which the inoculums followed 5% or 10% of medium. The conposition of BG-II medium can be seen in Table 1.

Table 1. Chemical composition of BG-II medium

Chemical	Stock solution (g/200 ml) for *Nostoc muscorum*	Stock solution (g/200 ml) for *Synechococcus elongatus*	Culture solution ADD: ml/L
$NaNO_3$	75 g/500 ml	12	10
$K_2HPO_4.H_2O$	8	4.8	1
$MgSO_4.H_2O$	15	15	1
$CaCl_2.H_2O$	7.2	7.2	1
Na_2CO_3	4	4	1
Citric acid	1.2	1.2	1
$Fe_2SO_4.H_2O$	1.2	1.2	1
EDTA	0.2	0.2	1
Trace elements	*	*	1
- H_3BO_3	2.68	2.68	1
- $MnCl_2.H_2O$	1.81	1.81	1
-$ZnSO_4.H_2O$	0.22	0.22	1
-$Na_2MoO_4.H_2O$	0.39	0.39	1
- $CuSO_4.H_2O$	0.079	0.079	1
- $Co(NO_3)_2.H_2O$	0.049	0.049	1

2. Assimilation of nitrogen-phosphate level of Cyanobacteria

Necessary of nitrogen-phosphate nutrients for *Nostoc muscorum* was determined through combining the different concentrations of NO_3 and HPO_4 contained in BG-II medium which was composited of 10 mL of $NaNO_3$ and 1 mL of K_2HPO_4. The concentrations of NO_3 and HPO_4 were categorized into several levels, namely:

a. 6 gram/200 mL of $NaNO_3$ stock solution containing 128.94 mg/L NO_3
b. 12 gram/200 mL of $NaNO_3$ stock solution containing 289.11 mg/L NO_3
c. 18 gram/200 mL of $NaNO_3$ stock solution containing 386.81 mg/L NO_3
d. 24 gram/200 mL of $NaNO_3$ stock solution containing 515.74 mg/L NO_3
e. 30 gram/200 mL of $NaNO_3$ stock solution containing 644.68 mg/L NO_3

The levels of HPO_4 were:

a. 1.6 gram/200 mL of K_2HPO_4 stock solution containing 19.41 mg/L HPO_4
b. 3.2 gram/200 mL of K_2HPO_4 stock solution containing 20.82 mg/L HPO_4
c. 4.8 gram/200 mL of K_2HPO_4 stock solution containing 22.27 mg/L HPO_4
d. 6.4 gram/200 mL of K_2HPO_4 stock solution containing 23.63 mg/L HPO_4
e. 8 gram/200 mL of K_2HPO_4 stock solution containing 25.16 mg/L HPO_4

The experimental design used for assimilation of nitrogen-phosphate is shown in Table 2.

Table 2. Experimental design used for assimilatio of nitrogen-phosphate in BG-II medium

Stock of K_2HPO_4	Stock of $NaNO_3$				
	128.94 mg/L NO_3 6 gram/200 mL	289.11 mg/L NO_3 12 gram/200 mL	386.81 mg/L NO_3 18 gram/200 mL	515.74 mg/L NO_3 24 gram/200 mL	644.68 mg/L NO_3 30 gram/200 mL
19.41 mg/L HPO_4 1.6 gram/200 mL	a b	a b	a b	a b	a b
20.82 mg/L HPO_4 3.2 gram/200 mL	a b	a b	a b	a b	a b
22.27 mg/L HPO_4 4.8 gram/200 mL	a b	a b	a b	a b	a b
23.63 mg/L HPO_4 6.4 gram/200 mL	a b	a b	a b	a b	a b
25.16 mg/L HPO_4 8 gram/200 mL	a b	a b	a b	a b	a b

The best growth of species in certain level of NO_3 and HPO_4 was observed through high biomass production in medium. Observation of biomass was done in last day of culture (day 7^{th}). Then biomass of *Nostoc muscorum* that had been harvested after 7 days in BG-II would be dried in 60°C for 24 hours. The dried biomass would be weighed and recorded. The best concentration of NO_3 and HPO_4 in BG-II medium would be used as main medium for next culture.

3. Evaluation of daily lipid content and biomass production

500 mL sample of *Nostoc muscorum* was taken daily from culture. The sample was filtrated for gaining the biomass. Then the biomass was dried in 60°C for 24 hours. The dried biomass would be used as sample of lipid content measurement and growth rate determination. Daily lipid content was observed through analyzing lipid production per day for 14 days. Lipid content of dried biomass was analyzed by Soxhlet method using soxhtec and petroleum ether as the solvent. The specific growth rate was calculated by the equation:

$$\mu = \frac{1}{t} \ln \frac{Biomass\ concentration\ at\ the\ end}{Biomass\ concentration\ at\ the\ initial}$$

Where, t : Duration of culture

Percentage of lipid would be calculated using the equation:

$$\%\text{Lipid} = \frac{(W3-W2)}{W1} \times 100\%$$

Where, W1 : Weight of sample (gram of dry weight)

W2 : Weight of cup

W3 : Weight of cup + lipid

RESULT AND DISCUSSION

In continous illumination condition at a rate of 2000 lux, *Nostoc muscorum* cultured in BG-II medium showed light green until dark green of cells color. The green color of *Nostoc muscorum* cells implied that this species contained photosynthetic pigment, namely chlorophyll-a. The shapes of cells were spherical and barrel-shaped, then the cells were arranged in filaments. In good condition of growth, *Nostoc muscorum* could form the sheaths of its colony and the sheaths could attach on the wall of culture glassware. They also developed heterocysts. According to Fogg *et al*. (1973), the function of heterocysts in *Nostoc* was as reproductive units which had ability to form germination of cells. The appearance of *Nostoc muscorum* colony and cells are shown in Figure 1.

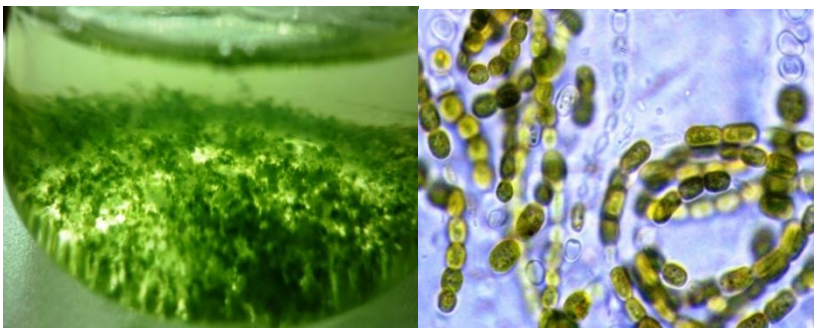

Figure 1. *Nostoc muscorum* colony and cells (100 x magnification)

Nitrogen-phosphate requirement of *Nostoc muscorum* in BG-II medium was determined by assimilating the nitrate (NO_3) concentration and phosphate (HPO_4) concentration which was combined in several levels to *Nostoc muscorum* for 7 days of cultivation. Determination of nitrogen-phosphate requirement was done to evaluate the exact necessary of *Nostoc muscorum* on nitrogen and phosphate nutrients for producing huge biomass. Based on result, the highest biomass production shown by Figure 2 and 3 below was in BG-II medium containing 644.68 mg/L of NO_3 (150 gram $NaNO_3$/L of stock solution) and 25.16 mg/L of HPO_4 (40 gram K2HPO4/L of stock solution).

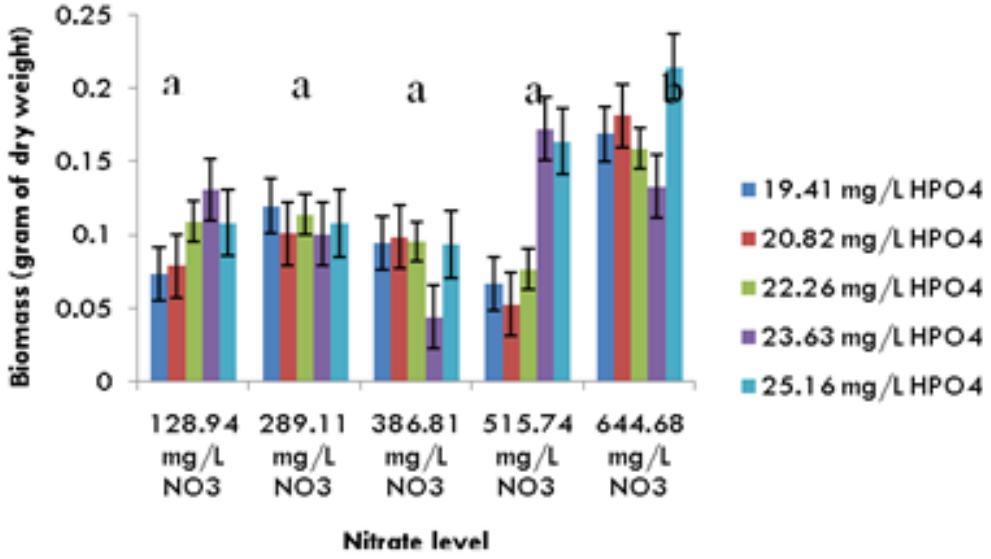

Figure 2. Determination of nitrate-phosphate requirement of *Nostoc muscorum* cultured in BG-II medium based on nitrate levels.

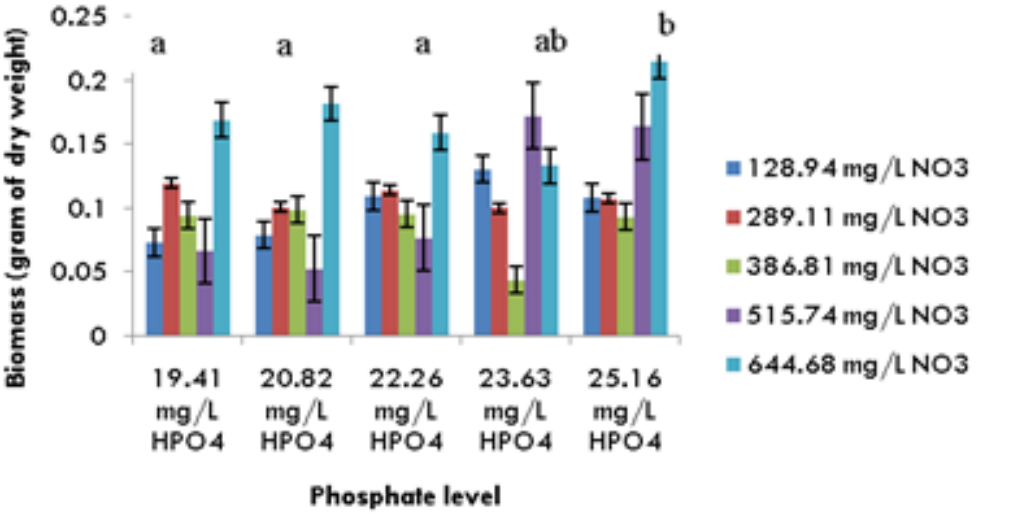

Figure 3. Determination of nitrate-phosphate requirement of *Nostoc muscorum* cultured in BG-II medium based on phosphate level

Nitrate requirement of *Nostoc muscorum* was gained in 644.68 mg/L of nitrate concentration with the highest biomass of *Nostoc muscorum* in amount of 0.21 grams/300 mL. Different levels of nitrate concentration significantly affected the biomass production of *Nostoc muscorum* ($p < 0.05$). Concentration at 644.68 mg/L of nitrate could encourage highest biomass production significantly compared to another levels of nitrate concentration. Continous light for 24 hours in *Nostoc muscorum* culture could effect on nitrate uptake for its growth. This experiment was in the same line with Han *et al*. (2013) revealed that the increment of biomass was gotten from 500 mg/L of initial $NaNO_3$ concentration which was in contrast with 200 mg/L of initial $NaNO_3$. Rai *et al*. (1981) explained that the uptake of nitrate was light dependent.

Su *et al*. (2012) also revealed the continuous illumination could result a higher biomass generation capability than could 12 hours illumination.

Phosphorus (P) is required nutrient for all organisms which is concentrated in the nucleic acids, lipid membranes, and ATP molecules of cells. The concentration of phosphate needed by *Nostoc muscorum* was less than needed nitrogen concentration, in which the ratio between nitrate/phosphate was 25.63/1 (by concentration). In this ratio of nitrate/phosphate, *Nostoc muscorum* could produce highest biomass. This result was in the same line with Hakanson *et al*. (2007) report that revealed Cyanobacteria could dominate the lake primary production at TN/TP ≤ 29/1 (by weight) and were much less abundant at higher ratios.

The highest biomass of *Nostoc muscorum* was achieved in highest level of phosphate concentration which was 25.16 mg/L of HPO_4. Different levels of phosphate concentration significantly affected the biomass of *Nostoc muscorum* ($p < 0.05$). High concentration of HPO_4 contained in BG-II medium caused high presence of phosphate ions which encouraged high absorption of those ions for P synthesis in cyanobacteria cells. Moreover, light intensity also participated to increase phosphate uptake of *Nostoc muscorum* in where continuous illumination resulted higher biomass generation. Based on Powell *et al*. (2009); Markou and Geogakakis (2011) that the luxury uptake of phosphorus was influenced by phosphate concentration, light intensity, pH, energy, and temperature.

Nostoc muscorum was cultured in phototrophic cultivation method with energy source from light and carbon source from inorganic carbon (Na_2CO_3). Generally total lipid of *Nostoc muscorum* during cultivation in BG-II for 14 days decreased day by day.

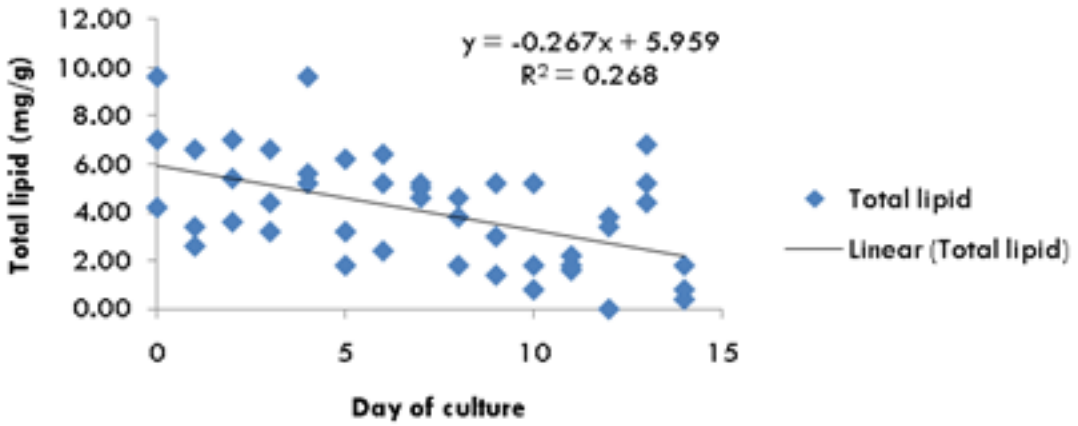

Figure 4. Total lipid of *Nostoc muscorum* cultured in BG-II medium

According to Figure 4 shown total lipid of *Nostoc muscorum* cultured in BG-II medium, the coefficient of decrement of total lipid during cultivation was 0.27 per day which followed the linear regression with the determination coefficient was 27%. Decreasing of total lipid could be caused by increment of cell growth to produce high biomass and activity of producing cell protein when the nitrate content was abundant in BG-II medium. Abundant nitrate in BG-II was a sufficient nitrogen condition for protein synthesis in the cells for their growth and carbon from

photosynthesis was channelled into energy and cell performance. *Nostoc muscorum* was achieving the exponential phase with continuous growth.

Amaro *et al.* (2011) explained the general principle of lipid formation was related to nitrogen extinction whereas inadequate nitrogen for protein synthesis required for growth causes the excess metabolite of carbon from photosynthesis. This metabolite is channelled into triacylglycerols or starch as storage molecules. Therefore, a common trend that higher lipid and carbohydrate content appeared with lower protein content. The evaluation of biomass production and total lipid of *Nostoc muscorum* cultured in BG-II medium is shown in Table 3.

Cultivation of *Nostoc muscorum* for 14 days in BG-II produced dried biomass at 0.95 g/L which continuously increased day by day with a slight decrease at day 10th of culture, however it was in contrast to lipid content and lipid production. As previous explaination, the decrement of lipid content was caused by cells growth of *Nostoc muscorum* and there was no opportunity to form acylglycerols as storage molecules in the cells. Furthermore, by considering biomass production and total lipid of *Nostoc muscorum* per day, day 4 could be expected as the good day for harvesting the cultivation. Biomass at 0.39 gram of dry weight/L and total lipid at 24.35 mg/g could result higher lipid production than another day.

Table 3. Evaluation of biomass production and total lipid of *Nostoc muscorum* cultured in BG-II medium

Day of culture	Biomass (g/L)	Total lipid (%)	Total lipid (mg/g)
0	0.25±0.00	3.36±0.72	33.62±7.24
1	0.24±0.01	1.39±0.02	13.92±0.23
2	0.32±0.00	2.11±0.07	21.09±0.70
3	0.31±0.03	1.26±0.39	12.56±3.89
4	**0.39±0.02**	**2.43±0.18**	**24.35±1.80**
5	0.41±0.03	1.29±0.42	12.90±4.19
6	0.42±0.00	1.31±0.11	13.12±1.15
7	0.42±0.02	1.15±0.03	11.5±0.33
8	0.54±0.03	0.77±0.06	7.69±0.59
9	0.76±0.11	0.39±0.07	3.91±0.73
10	0.35±0.05	0.33±0.33	3.27±3.30
11	0.37±0.07	0.40±0.02	3.96±0.17
12	0.43±0.12	0.87±0.19	8.73±1.88
13	0.67±0.01	0.84±0.08	8.36±0.76
14	0.95±0.06	0.07±0.02	0.66±0.20

The comparison of biomass production to total lipid was inversely proportional occurred in *Nostoc muscorum*. While biomass production increased dramatically, total lipid was declined. The explaination about this study could be related to study which was done by Klok *et al.* (2013). There was a linear positive relation between nitrogen consumption rate and specific growth rate of microalgae. Whereas, *Nostoc muscorum* was cultured in high nitrate and phosphate content, such 644.68 mg/L of NO_3 and 25.16 mg/L of HPO_4. High nitrate concentration in culture medium encouraged high consumption rate of nitrate by *Nostoc muscorum*. High phosphate content also supported the growth of this species beside arranging the structural P synthesis in the cells. Finally, increment of biomass of *Nostoc muscorum* was shown through high specific growth rate of it. Decreasing of total lipid in this study implied that

Nostoc muscorum was still growing for 14 days of culture through consume nitrate and phosphate which were still abundant. Its growth produced its high biomass and protein content in the cells.

The specific growth rate of *Nostoc muscorum* cultured for 14 days was 0.10 µ/day. Reproduction of *Nostoc muscorum* was done through vegetative reproduction by fragmentation of the filament and sometimes asexually by resting cells (spores) called akinetes. According to Thompson *et al*. (2009), akinetes played in cyanobacterial bloom dynamics especially within water bodies with varying light quality and it previously be known that akinetes were affected by light, nutrients, and temperature.

BG-II medium provided good nutrition especially phosphate for *Nostoc muscorum* to develop its akinete and heterocyst cells. Controlled light and temperature participated in formation of those structural cells. However, formation of akinete and heterocyst cells could need more time and for every filament consisted of many cells only could form limited akinete and heterocyst cells.

CONCLUSION

Evaluation of the nutrient requirement and daily lipid production of *Nostoc muscorum* cultured in BG-II medium showed that *Nostoc muscorum* required nitrate (NO_3^-) at rate of 644.68 mg/L and phosphate (HPO_4^-) at rate of 25.16 mg/L. The highest lipid content was reached up in day 4 of culture that was 24.35 mg/g (2.43 %). Biomass production of *Nostoc muscorum* cultured in BG-II medium for 14 days could grow 0.10 µ/day. *Nostoc muscorum* has good potential as biodiesel feedstock through producing high biomass in BG-II medium.

REFERENCES

Amaro H.M., Guedes A.C., Malcata F.X. 2011. Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy (88)*, 3402-3410 p.

Atabani A.E., Silitonga A.S., Badruddin I.A., Mahlia T.M.I., Masjuki H.H., Mekhilef S. 2012. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable and Sustainable Energy Reviews (16)*, 2070-2093p.

Demirbas A. 2007. Importance of biodiesel as transportation fuel. *Energy Policy (35),* 4661-4670 p.

EIA. 2012. Short term energy outlook. http://www.eia.gov/forecasts/steo/archives/May12.pdf [May 15th 2012].

Fogg G.E, Stewart W.D.P, Fay P, Walsby A.E. 1973. *The Blue-Green Algae*. New York: Academic Press.

Hakanson L, Bryhn A.C, Hytteborn J.K. 2007. On the issue of limiting nutrient and predictions of cyanobacteria in aquatic systems. *Science of the Total Environment (379)*, 89-108 p.

Han F, Huang J, Li Y, Wang W, Wan M, Shen G, Wang J. 2013. Enhanced lipid productivity of *Chlorella pyrenoidosa* through the culture strategy of semi-continuous cultivation with nitrogen limitation and pH control by CO_2. *Bioresource Technology* [Accepted manuscript].

Jayed M.H. Masjuki H.H., Kalam M.A., Mahlia T.M.I., Husnawan M., Liaquat A.M. 2011. Prospects of biodiesel engine vehicles in Malaysia and Indonesia. *Renewable and Sustainable Energy Reviews (15)*, 220-235 p.

Klok A.J, Martens D.E, Wijffels R.H, Lamers P.P. 2013. Simultaneous growth and neutral lipid accumulation in microalgae. *Bioresource Technology (134)*, 233-243 p.

Malakar E, Goswani R.C.D, Kalita M.C. 2012. A study on two potential BGA isolates *Cylindrospermum majus* and *Nostoc muscorum* of Assam, North-East India. *Archives of Applied Science Research 4(1),* 487-496 p.

Markou G. and Georgakakis D. 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewater: a review. *Applied Energy (88)*, 3389-3401 p.

Parmar A., Singh N.K., Pandey A., Gnansounou E., Madamwar D. 2011. Cyanobacteria and microalgae: a positive prospect for biofuels. *Bioresource Technology (102),* 10163-10172 p.

Powell N, Shilton A, Chisti Y, Pratt S. 2009. Towards a luxury uptake process via microalgae-defining the polyphosphate dynamics. *Water Research (43)*, 4207-4213.

Rai A.K, Kashyap A.K, Gupta S.L. 1981. ATP-dependent uptake of nitrate in Nostoc muscorum and inhibition by ammonium ions. *Biochimica et Biophysica Acta (BBA)-General Subjects 674(1)*, 78-86 p.

Shalaby E.A. 2011. Algal biomass and biodiesel production. In M. Stoytcheva and G. Montero (Eds). *Biodiesel-Feedstock and Processing Technologies* (pp111-132). InTech: Croatia.

Singh J. and Gu S. 2010. Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews (14)*, 2596-2610 p.

Su Y, Mennerich A, Urban B. 2012. Coupled nutrient removal and biomass production with mixed algal culture: impact of biotic and abiotic factors. *Bioresource Technology (118)*, 469-476 p.

Thompson P.A, Jameson I, Blackburn S.I. 2009. The influence of light quality on akinete formation and germination in the toxic cyanobacterium *Anabaena circinalis*. *Harmful Algae (8)*, 504-512 p.