The International Journal of Biotechnology
2013 Vol.2, No.1, pp.42-51
ISSN(e): 2306-6148
ISSN(p): 2306-9864

HETEROTOPHIC GROWTH OF *ANKISTRODESMUS* SP. FOR LIPID PRODUCTION USING CASSAVA STARCH HYDROLYSATE AS A CARBON SOURCE

Mohamad Agus Salim[1]

[1]*Biology Department, Faculty of Science & Technology, State Islamic University of Sunan Gunung Djati Bandung Jl. A. H. Nasution No.105 Bandung, Indonesia*

ABSTRACT

Research has been done on the culture of Ankistrodesmus sp. through heterotrophic conditions to obtain lipid content. Heterotrophic culture with the addition of several concentrations of cassava starch hydrolysate (CSH) as a source of organic carbon that increased lipid content. The highest cell density and cell growth rate (cell division) of Ankistrodesmussp achieved at 10 g.l^{-1} of CSH concentration on 2.46 x 10^{6} cells.ml^{-1} which occurred at the 8th day and 0.50 cells.day^{-1} respectively. While the highest biomass and lipid content achieved at 5 g.l^{-1} of CSH concentration on 0.94 g.l^{-1} and 26% respectively.

Keywords: *Ankistrodesmus*sp, Cassava starch hydrolysate, Heterotrophic, Lipid content.

INTRODUCTION

Due to excessive and increasing combustion of fossil fuel, the amount of greenhouse gas CO_2 has increased. About 80% of this energy demand is delivered from fossil fuels with the consequence of an increase of greenhouse gas emissions in the atmosphere that provokes serious climate changes by global warming. As a result global warming and climate change are threatening ecological stability, food security and social welfare and this will lead to more environmental damage (*1*). A constant rising worldwide demand, furthermore the fossil fuels supplies are constantly diminishing has motivated the scientists and technologists to think about alternative energy sources. In the recent years much thrust has been put on to examine the possibilities of using microalgae as a source of oil for energy applications (*2*). Microalgal oil for biodiesel utilization can be produced up to 7-31 times more than soy oil and palm oil (*3*). A higher oil content indicates a higher fatty acid content of microalgae, the more potential of microalgae to be able to produce

biodiesel (4). *Ankistrodesmus* sp. is one species of unicellular green microalgae to produce oil. *Ankistrodesmus* sp. cell containing fatty acids can be used as a base for the production of biodiesel (5). To increase lipid content of microalgae, it needs proper cultures, that is by manipulating the environmental factors such as light, CO_2, temperature, pH, and nutrient media (6).The heterotrophic condition can provide microalgae to survive if there is an energy supplied to cell growth. In heterotrophic condition microalgae could not perform photosynthesis but glycolysis process, so that it need supplied from glucose or other simple compounds. Glucose as an organic carbon source, and its use as an energy source by microalgae through the process of glycolysis, which will be used for the manufacture of lipids, carbohydrates and proteins (7).

A study by (8) indicates heterotrophic microalgae culture with the addition of corn starch hydrolysate in a concentration of 10 $g.l^{-1}$ as a source of carbon and nutrients for growth of the microalgae *Chlorella protothecoides*, the total lipid content can reach 55.2%. Likewise research (9) the accumulation of lipid content by *C. protothecoides* can reach up to four times in heterotrophic condition than autotrophic condition. Lipid productivity of microalgae is important along with the increasing demand for bioenergy source. Therefore, the main objectives of this study are directed at optimization of lipid production of microalgae *Ankistrodesmus*sp. cultured heterotrophic condition with the addition of cassava starch hydrolysate.

MATERIALS AND METHODS

Microalgae Production

Microalgae *Ankistrodesmussp* was derived from the collection of the Laboratory of Biology Department, Faculty of Science and Technology of State Islamic University SunanGunungDjati Bandung, Indonesia was the result of isolation from the fresh water Cibiru, Bandung. In the experiments, *Ankistrodesmussp*was cultivated in 500 ml Erlenmeyer flask with 200 ml working volume of Basal Bold medium, under 25 ±1 ^oC and 180 µmol $m^{-2}s^{-2}$. light intensity was measured by a light meter. For creating heterotrophic condition, every Erlenmeyer flask were covered by carbon paper, meanwhile for autotrophic condition as control without covering by carbon paper. The initial density of cell inoculum was 10.000cells.ml^{-1} and the initial pH was 6.5. Cultures were aerated continuously with filtered (0.22 µm) mixtures via bubbling from the above of Erlenmeyer flask with an aeration rate of 200 $ml.min^{-1}$ (i.e., 0.25 vvm, volume gas per volume media per minute). Different concentrations of cassava starch hydrolysate were added to the basal bold medium.

Measurement of Growth Rate

Growth was evaluated over time in terms of optical density (OD) (540 nm) (Hitachi U-2000), spectrophotometer), DW (dry weight) (Whatman GF/C 1.2 µm). All samplings were performed at least in duplicate. The dry cell weight ($g.l^{-1}$) was measured according to the method as described by (*10*). Microalgal cells were harvested by centrifugation (5804R, Eppendorf, Germany) at 8000 rpm for 5 min and washed twice with distilled water. The microalgal pellet was lyophilized drying in a freeze drier (FD-1-50, Boyikang, China) for dry weight measurement.

Oil Extraction

The total lipids were extracted from microalgal cells using a modified method of (*11*). Dry microalgal cells were pulverized in a mortar mixed with liquid nitrogen and extracted using mixture of ethanol: hexana (1:1 v/v). About 5 ml solvents and 100 mg dry cell were used in each extraction step. The procedure was repeated three times until the total lipids were fully extracted. The solvent phase was transferred by pipette and evaporated in a rotary evaporator under vacuum at 60°C. Then the total lipids were weighed using analytical balance (BS 124S, Sartorius, Germany).

Statistical Analysis

The mean value of all datas were compared by variances analysis (ANOVA) and then Duncan'sMultiple RangeTest (DMRT) for pair wise comparison was used at the 5% significance level (*12*). The growth rate of microalgae *Ankistrodesmus* sp. was measured by quadratic regresion equation to obtain value of cell division per day.

RESULTS AND DISCUSSION

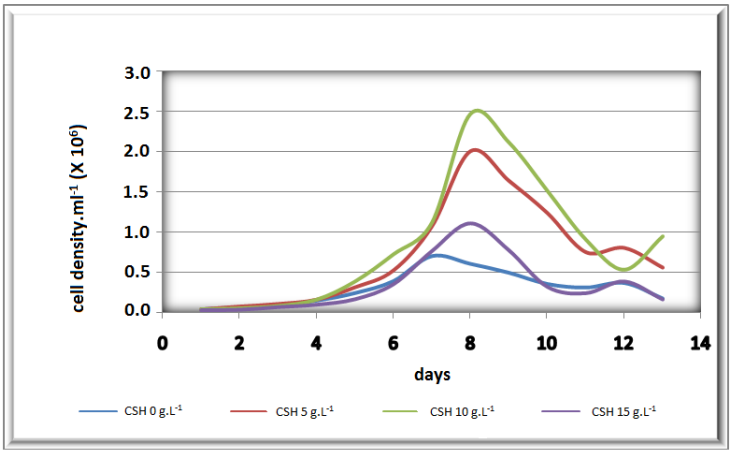
Cell Densities of *Ankistrodesmus*sp

Observations to growth pattern of *Ankistrodesmus* sp. on the treatment of many CSH concentrations indicated two peaks of growth (Figure 1.). In heterotrophic *Ankistrodesmus*sp culture, the highest cell density of 2.47 x 10^6 $cells.ml^{-1}$ has achieved by the treatment of 10 $g.l^{-1}$ CSH concentration on the first peak of growth at the 8^{th} day, while the second peak of growth was occurred on the 13^{th} day with the cell density of 0.95 x 10^6 $cells.\ ml^{-1}$. The pattern of growth in treatment of CSH concentrations of 0 $g.l^{-1}$ (control) reached the first peak of growth occurring on the 7^{th} day with cell density of 0.70 x 10^6 $cells.ml^{-1}$, whereas the second peak of growth occurred on the 12^{th} day with cell density of 0.37 x 10^6 $cells.ml^{-1}$. In the treatment of CSH concentration 5 $g.l^{-1}$, reached the first peak of growth on the 8^{th} day with cell density of 2.00 x 10^6 $cells.ml^{-1}$, and the second peak of growth occurred on the 12^{th} day with cell density of 0.80 x 10^6 $cells.ml^{-1}$. At the treatment of CSH concentration of 15 $g.l^{-1}$, reached the first peak of growth on the 8^{th} day

with cell density of 1.11 x 10^6 cells.ml^{-1}, whereas the second peak of growth occurred on the 12th day with a cell density of 0.39 x 10^6 cells.ml^{-1}.

The first peak of growth *Ankistrodesmus*sp was the maximum cell density, then the pattern of growth declined and rose again to reach the top of the second peak of growth but the cell density was lower than in the first peak of growth and down again which finally reaches death or death phase.

Figure-1.cell density of microalgae *Ankistrodesmus*sp. in heterotrophic culture with various treatment of CSH concentrations.



The Growth Rate of *Ankistrodesmus* sp

Based on the hyperbolic curve with a quadratic regression equation during 13 days on heterotrophic culture, the maximum growth rate of *Ankistrodesmus* sp achieved by treatment of CSH concentration 5 g.l^{-1} and 10 g.l^{-1} with cell division 0.49 cell.day^{-1} and 0.50 cell.day^{-1} occurred on the 6.54th day (Figure 2.) and the 6.65th day (Figure 3.) respectively. While the treatment of CSH concentration of 0 g.l^{-1} achieved cell division 0.41 cell.day^{-1} on the 5.60th day (Figure 4.), and the concentration of 15 g.l^{-1} achieved cell division 0.41 cell.day^{-1} on the 7.23th day (Figure 5.). As reported by (*13*) microalgal cells would be divided up to 3 times daily cleavage. Cell of microalgae obtaining adequate nutrition would be able to grow well.

Figure-2. The relative of growth rate of microalgae *Ankistrodesmus* sp. with the addition of CSH 0 g.l^{-1} in the BBM for 13 days. (Regresion equation y = 0.226 + 0.067 x - 0.006 x^2)

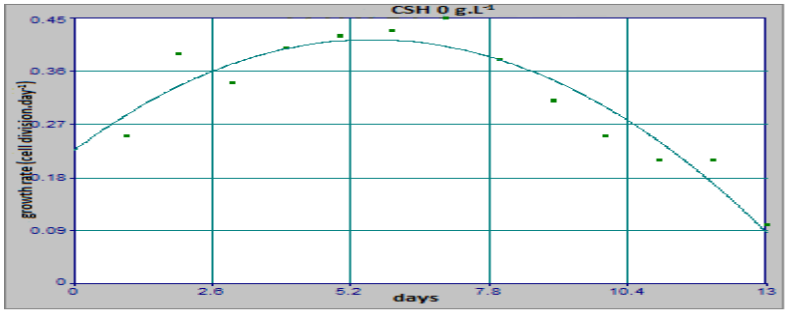


Figure-3. The relative of growth rate of microalgae *Ankistrodesmus* sp. with the addition of CSH g.l^{-1} in the BBM for 13 days. (Regresion equation y = 0.171 + 0.099x – 0.007x^2)

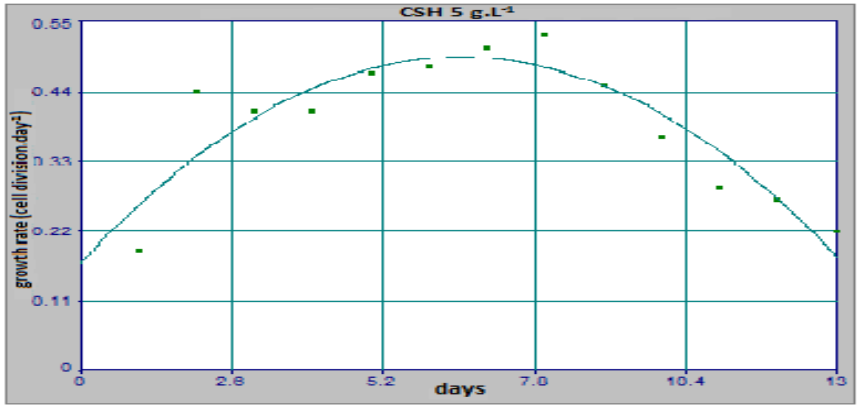


Figure-4. The relative of growth rate of microalgae *Ankistrodesmus* sp. with the addition of CSH 10 g.l^{-1} in the BBM for 13 days. (Regresion equation y = 0.177 + 0.098x –0.007x^2)

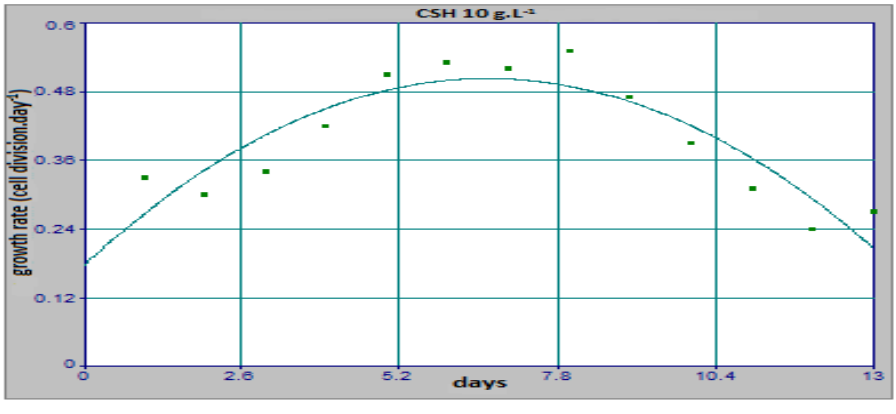
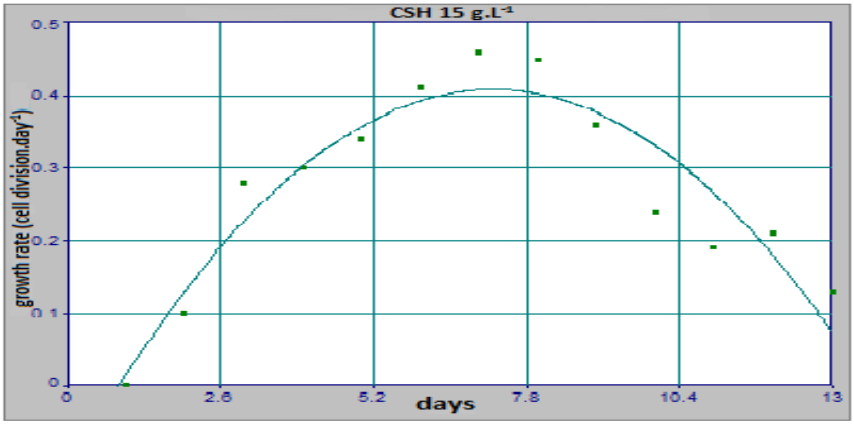


Figure-5. The relative of growth rate of microalgae *Ankistrodesmus* sp. with the addition of CSH 15 g.l^{-1} in the BBM for 13 days. (Regresion equation y = -0.118 + 0.0145x – 0.010x^2)



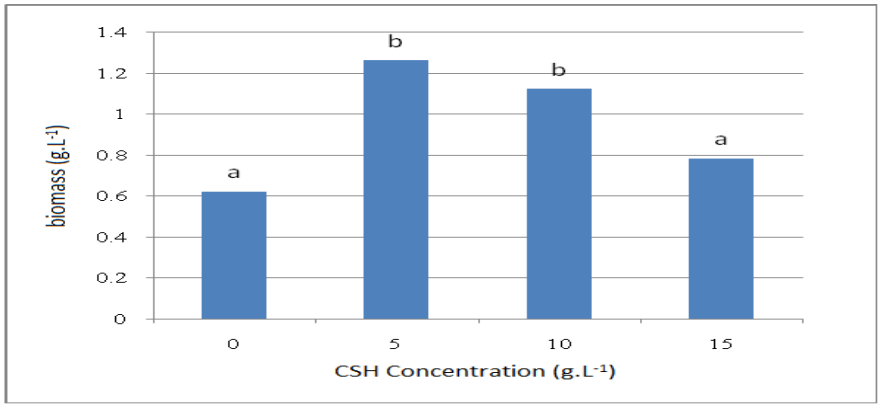
In each treatment of CSH concentration of 0, 5 and 10 g.l^{-1} (Figure 2, 3 and 4.) no adaptation was found. This cases occured due to media of treatment (MBB) was relatively equal to the previous maintenance media. (*14*)found that the microorganisms living in treatment and maintenance medium have the same number of cells as it has the same environmental conditions, causing adaptation phase did not appear that the cell more quickly into the exponential phase.In the treatment of CSH concentration of 15 g.l^{-1} indicated a phase of adaptation (Figure 5.), Because the treatment media was not the same as the maintenance media. Culture medium with the addition of 15 g.l^{-1} CSH had physical and chemical properties that very different from the maintenance medium.

Biomass of *Ankistrodesmus*sp

Based on the results of analysis of variance, the concentration of CSH as a source of organic carbon and energy for growth in heterotrophic culture of microalgae *Ankistrodesmus* sp. indicated significantly different (F> 0.05). This suggests that induction of different concentration of CSH could affect the growth (*15*).

Further results could be seen in Figure 6, that the treatment of CSH concentration of 5 g.l^{-1} and 10 g.l^{-1} could result the biomass of 1.26 g.l^{-1} and 1.12 g.l^{-1} respectively. (*16*) stated that optimum concentration of organic substances were required for healthy algal culture, besides the algal culture should be pure when cultured.

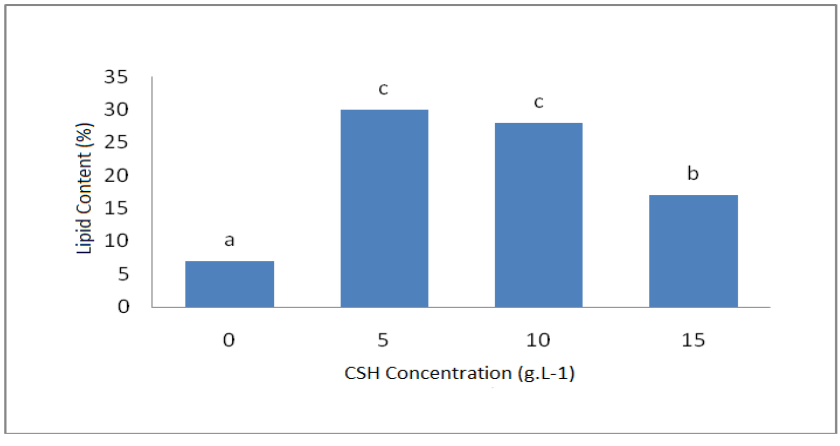
Figure-6. The effect of CSH to biomass of *Ankistrodesmus*sp (numbers followed by different letters indicate difference at 5% level test).



Lipid Content of *Ankistrodesmus*sp

Based on the results of variance analysis, CSH concentrations could affect the total production of lipids. In the figure 7 could be seen that the optimum CSH concentrations were 5 $g.l^{-1}$ and 10 $g.l^{-1}$ with lipid content of 30% and 28%.

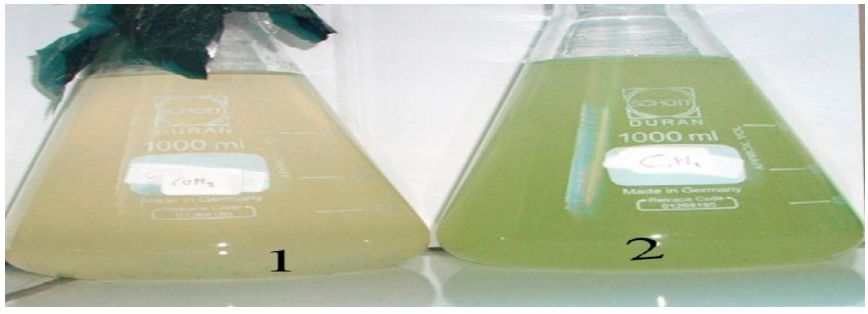
Figure-7. The effect of CSH to lipid contents of *Ankistrodesmus* sp. (numbers followed by different letters indicate difference at 5% level test).



(*17*) stated that the optimum concentration of organic substrates required for microalgal culture. If the concentration did not fix then there were likely competition or contamination with other species of microalgae. In addition to the proper concentration of organic substrates, pure of microalgae cultures were also required to avoid contamination. Heterotrophic culture also resulted in chlorophyll content of microalgae was reduced (Figure 8.). (*18*) stated that microalgal cell in heterotrophic culture would be contained droplet of oils in yellow colored body, while in autotrophic culture would be contained much more chlorophyll pigment. (*9*) stated that the culture of heterotrophic microalgae *C. prototechoides* resulted in lipid storage in the body so that

the body color was yellow, while in autotrophic culture colored green because of excessive chlorophyll content.

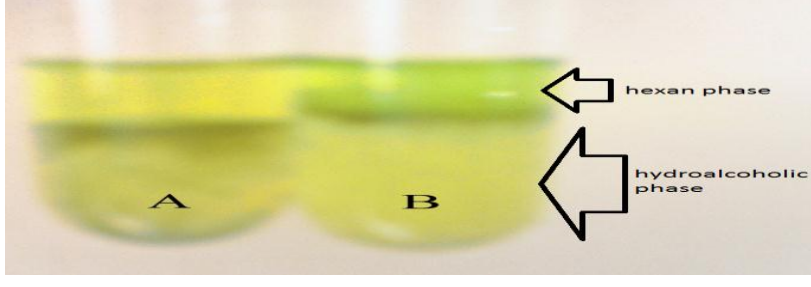
Figure-8. The Culture of microalgae *Ankistrodesmus* sp.
A1: heterotrophic culture ; A2: autotrophic culture



Color of the extracted lipids from heterotrophic culture were also different from the autotrophic culture (Figure 9). Lipids from autotrophic culture was green due to chlorophyll pigment gather extracted by ethanol solvent (*19*). In heterotrophic culture, lipids extracted was yellow, because the lack of chlorophyll pigment (*9*).

Figure-9. The lipids extraction of microalgae *Ankistrodesmus*sp with solvent ethanol : hexana (1 : 1)

(A= heterotrophic culture, B= autotrophic culture)



CONCLUSION

Heterotrophic cultivation had drawn increasing attention and it was regarded as the most practical and promising way to increase the productivity. Microalgae could adapt to different organic substances including cassava starch hydrolysate after acclimatization. The highest cell density and cell growth rate (cell division) of *Ankistrodesmus*sp achieved at 10 $g.l^{-1}$ of CSH concentration on 2.46 x 10^{6} $cells.ml^{-1}$ which occurred at the 8^{th} day and 0.50 $cells.day^{-1}$

respectively. While the highest biomass and lipid content achieved at 5 $g.l^{-1}$ of CSH concentration on 0.94 $g.l^{-1}$ and 26% respectively.

ACKNOWLEDGEMENT

Authors are thankful to Laboratory of Microalgal Technology of Biology Departement of Science & Technology Faculty for providing facilities to carry out the research work. Authors are thankful to Mr. Nano Sumarno for his help in carrying out the research work. All the financial assistance for the research work were provided by LembagaPenelitian (Lemlit) State Islamic University SunanGunungDjati Bandung, Indonesia.

REFERENCES

1. Prabakaran, P., and Ravindran, A. D. (2012) Influence of different carbon and nitrogen sources on growth and CO2 fixation of microalgae, *Advances in Applied Science Research3*, 1714-1717.
2. Schlagermann, P., Gottlicher, G., Dillschneider, R., Rosello, S., R. , and Posten, C. (2012) Composition of algal oil and its potential as biofuel, *Journal of Combustion2012*, 14.
3. Singh, J., and Sai, G. (2010) Commercialization potential of microalgae for biofuels production, *Renewable and Sustainable Energy Reviews14*, 2596–2610.
4. Sharma, K. K., Schuhmann, H., and Schenk, P. M. (2012) High lipid induction in microalgae for biodiesel production, *Energies5*, 1532-1553.
5. Sudhakar, K., and Premalatha, M. (2012) Microalgal technology for sustainable energy production, *Journal of Sustainable Energy & Environment3*, 59-62.
6. Zuka, Z., McConnell, B., and Farag, I. (2012) Comparison of freshwater and wastewater medium for microalgae growth and oil production, *Journal of American Science8*, 392 -398.
7. Goswami, R. C. D., Kalita, N., and Kalita, M. C. (2012) A study on growth and carbon dioxide mitigation by microalgae Selenastrum sp.: its growth behavior under different nutrient environments and lipid production, *Annals of Biological Research3*, 499-510.
8. Lu, Y., Zhai, Y., Liu, M., and Wu, Q. (2010) Biodiesel production from algal oils using cassava (Manihot esculenta Crantz) as feedstock, *J Appl. Phycol22*, 573–578.
9. Miao, X. L., and Wu, Q. Y. (2005) Biodiesel production from heterotrophic microalgal oil, *Bioresour Technol97*, 841–846.

10. Chiu, S. Y., Tsai, M. T., Kao, C. Y., Ong, S. C., and Lin, C. S. (2009) The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal, *Eng. Life Sci9*, 254–260.
11. Zhu, L., Zhang, X., Ji, L., Song, X., and Kuang, C. (2007) Changes of lipid content and fatty acid composition of Schizochytrium limacinum in response to different temperatures and salinities, *Process Biochem42*, 210-214.
12. Gomez, K. A., and Gomez, A. A. (1995) *Statistical prosedures for agriculture research*, University of Indonesia (UI-Press), Jakarta.
13. Salih, F. M., and Haase, R. A. (2012) Potentials of microalgal biofuel production, *Journal of Petroleum Technology and Alternative Fuels3*, 1-4.
14. Wang, H., Fu, R., and Pei, G. (2012) A study on lipid production of the mixotrophic microalgae Phaeodactylum tricornutum on various carbon sources, *African Journal of Microbiology Research6*, 1041-1047.
15. Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., and Ruan, R. (2009) Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant, *Appl Biochem BiotechnolDOI 10.1007/s12010-009-8866-7*.
16. Abubakar, L. U., Mutie, A. M., Kenya, E. U., and Muhoho, A. (2012) Characterization of algae oil (oilgae) and its potential as biofuel in Kenya, *Journal of Applied Phytotechnology in Environmental Sanitation1*, 147-153.
17. Shah, D. (2012) Effect of glucose supplementation on nigttime biomass loss and productivity of microalgae Chlorella, submitted in partial fulfillment of requirements for the degree, *Masters of Science in Chemical Engineering at The Cleveland State University*.
18. Xu, H., Miao, X. L., and Wu, Q. Y. (2006) High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters, *J. Biotechnol126*, 499–507.
19. Chi, Z., Pyle, D., Wen, Z., Frear, C., and Chen, S. (2007) A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgalfermentation, *Process biochem42*, 1537-1545.