Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Effects of organic carbon source and light-dark period on growth and lipid accumulation of *Scenedesmus* sp. AARL G022

Doungpen Dittamart [1], Chayakorn Pumas [1, 2], Jeeraporn Pekkoh [1, 2] and Yuwadee Peerapornpisal [1, 2, *]

[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200 Thailand
[2] Science and Technology Research Institute, Chiang Mai University, Chiang Mai, 50200 Thailand

* Corresponding author, e-mail: yuwadee.p@cmu.ac.th; tel: +66 (0)53 941950 ext.119; fax: +66 (0)53 852 259

Received: 8 March 2013 / Accepted: 4 August 2014 / Published: 13 August 2014

Abstract: The levels of different organic carbon supplements in a mixotrophic culture were optimised to enhance biomass and lipid accumulation in *Scenedesmus* sp. AARL G022. The supplement nutrients, viz. glucose, glycerol and sodium acetate, were compared with non-organic carbon supplement (photoautotrophic culture). The most suitable carbon source was found to be 0.05M glucose, giving a yield of 2.78 ± 0.86 $g.L^{-1}$ of biomass and 233.68 ± 35.34 $mg.L^{-1}$ of crude lipid. The highest yield of biomass (4.04 ± 0.36 $g.L^{-1}$) was obtained from a light-dark cycle of 24:0 hr. The highest crude lipid yield of 396.35 ± 11.60 $mg.L^{-1}$ was obtained from a light-dark cycle of 16:8 hr. The optimised condition for culturing *Scenedesmus* sp. AARL G022 is to cultivate it under a mixotrophic condition using 0.05M of glucose supplement with a light-dark cycle of 16:8 hr.

Keywords: *Scenedesmus* sp. AARL G022, algal bio-oil, mixotrophic cultivation

INTRODUCTION

Algal biofuels are generating considerable interest around the world. Microalgae grow faster than terrestrial plants, frequently doubling their biomass in one day, and can accumulate lipids to as high as 50% of the cell dry weight and express less demand for arable land [1-3]. These lipids can be converted into biodiesel by a chemical process called transesterification and this biodiesel can be used directly or as a blend with diesel fuel for diesel engines [4, 5]. Hence microalgal biomass has the potential to be used as raw material for bio-oil production and it has thus been widely recognised as feedstock for third-generation biofuels [1]. However, it is difficult to cultivate and harvest these

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

algae in a cost-efficient manner. To achieve economic feasibility of this process, the algal biomass and its lipid content have to be increased and the operation cost has to be reduced [6]. Many factors affect the biomass and lipid productivity of microalgae, for example nitrogen source and concentration [7], light intensity [8], light-dark period [9] and trophic condition [6]. Some microalgae can use organic carbon sources as well as carbon dioxide for growth under mixotrophic conditions, in which the microalgae obtain energy from photosynthesis and oxidation of organic compounds. This indicates that the microalgae are able to live under either photoautotrophic or chemoheterotrophic conditions, or both. The CO_2 released by microalgae via respiration can be trapped and reused under photoautotrophic cultivation [10]. Therefore, it is evident that mixotrophic cultivation can shorten the growth cycle and increase biomass yield more efficiently than can photoautotrophic or chemoheterotrophic culture, separately or combined [11]. Hence a higher growth rate and lipid accumulation in microalgae are promoted [12].

Glucose has normally been used as carbon source in mixotrophic culture but other carbon sources such as crude glycerol from the biodiesel industry, sugars from industrial or agricultural wastes and cane molasses are also found to be promising for the cultivation of mixotrophic algae.

Scenedesmus sp. AARL G022 has recently been reported as a fast growing strain whose large cells are easily harvested and tolerant towards contamination. However, the lipid content in this alga was not so high when compared with other microalgae [13]. For this reason, this research is aimed to analyse the effects of organaic carbon (glucose, glycerol and sodium acetate) and light-dark cycle on the growth and lipide accumulation of *Scenedesmus* sp. AARL G022 grown under laboratory conditions.

MATERIALS AND METHODS

Culture and Growth Conditions

The axenic culture of *Scenedesmus* sp. AARL G022 was obtained from the culture collection of Applied Algal Research Laboratory, Department of Biology, Chiang Mai University. The alga was isolated from a natural water source in Chiang Mai. It was morphologically identified under a compound microscope and maintained in 100 mL of algal medium [14] on an orbital shaker at 25° under constant illumination with a fluorescent lamp (10.8 $\mu moles.m^{-2}.s^{-1}$).

The cultivation was performed in a flask containing 700 mL of the medium supplemented with glucose, glycerol or sodium acetate as the organic carbon source. Each of the carbon sources was supplied in three concentrations: 0.01, 0.02 and 0.05 M. Each experiment was conducted in triplicate. The culturing was performed in a temperature-controlled incubator at 25±2° under continuous illumination with fluorescent lamp (10.8 $\mu moles.m^{-2}.s^{-1}$). The growth was spectrophotometrically measured at optical density of 665 nm and the cells were counted on a haemacytometer counting chamber under a compound microscope (Olympus Normaski) every two days. When each culture reached the stationary phase, the cells were harvested by centrifugation and dried at 60° for 48 hr. The change in cell morphology was observed under the compound microscope and recorded.

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

Effect of Light-Dark Period

To study the effect of photo-period on growth and lipid accumulation, *Scenedesmus* sp. AARL G022 was grown in a temperature controlled incubator at 25±2° under photoautotrophic (continuous illumination), chemoheterotrophic (no illumination) and mixotrophic conditions with light-dark cycles of 8:16, 12:12, 16:8 and 24:0 hr. In the chemoheterotrophic and mixotrophic cultures, 0.05M glucose was supplied as the organic carbon source.

Biomass Yield and Lipid Extraction

To measure the biomass yield, the microalgal culture (20 mL) was filtered using a pre-weighed GF/C filter paper (Whatman) and dried in an oven at 60° until a constant weight was reached (approximately 2-3 days). The biomass obtained was expressed in grams per litre ($g.L^{-1}$).

The algal lipid content was analysed following the protocol of Bligh and Dyer [15]. Dry algal biomass (approximately 0.1 g) was soaked in 15 mL of chloroform: methanol (2:1 v/v) at room temperature for 24 hr. The resulting solution, after being separated by centrifugation at 6,000 rpm for 10 min. to remove cell sludge, was added with 0.9% NaCl solution (5 mL), allowed to stand for a few minutes and the organic phase was collected. The organic solvent was then evaporated, the remaining crude lipid was dried at 50° and the weight of the lipid was obtained and expressed as per cent of dry biomass and millgrams per litre of the culture.

Statistical Analysis

Statistical comparison between the groups was done by one-way analysis of variance (ANOVA) and Duncan's multiple-range test, using SPSS for Windows version 14.0. The *p*-values that were less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of Organic Carbon

It was observed that under mixotrophic conditions, *Scenedesmus* sp. AARL G022 showed a higher growth rate than that under photoautotrophic conditions (control). The optical density and cell concentration under different concentrations of glucose supplement are demonstrated in Figures 1A and 1B. The glucose supplement enhanced both the number of cells and the OD_{665}. The highest growth rate was observed with 0.05M glucose, followed by 0.02M, 0.01M and non-glucose supplement (photoautotrophic control) respectively. The highest biomass of 2.78±0.86 $g.L^{-1}$ was observed with 0.05M glucose supplement and was 17 times higher than control (Table 1). This result indicates that the stationary growth rate was due to the limitation of carbon source and thus the growth performance would increase with increase in the substrate concentration. According to a previous study, microalgae could be cultivated under glucose up to 0.17M [16]. However, it needs to be noted that a high glucose supplement could inhibit microalgal growth [17] as well as increase the capital cost.

Figures 1C and 1D show the cultures supplemented with glycerol at different concentrations. It is interesting to note that the number of cells from all glycerol concentrations and from the photoautotrophic control was not different, although the highest OD_{665} of 0.41 was obtained when the cells were cultured with the 0.01M glycerol supplement. In contrast, the 0.05M glycerol supplement gave the highest biomass and lipid content (Table 1).

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

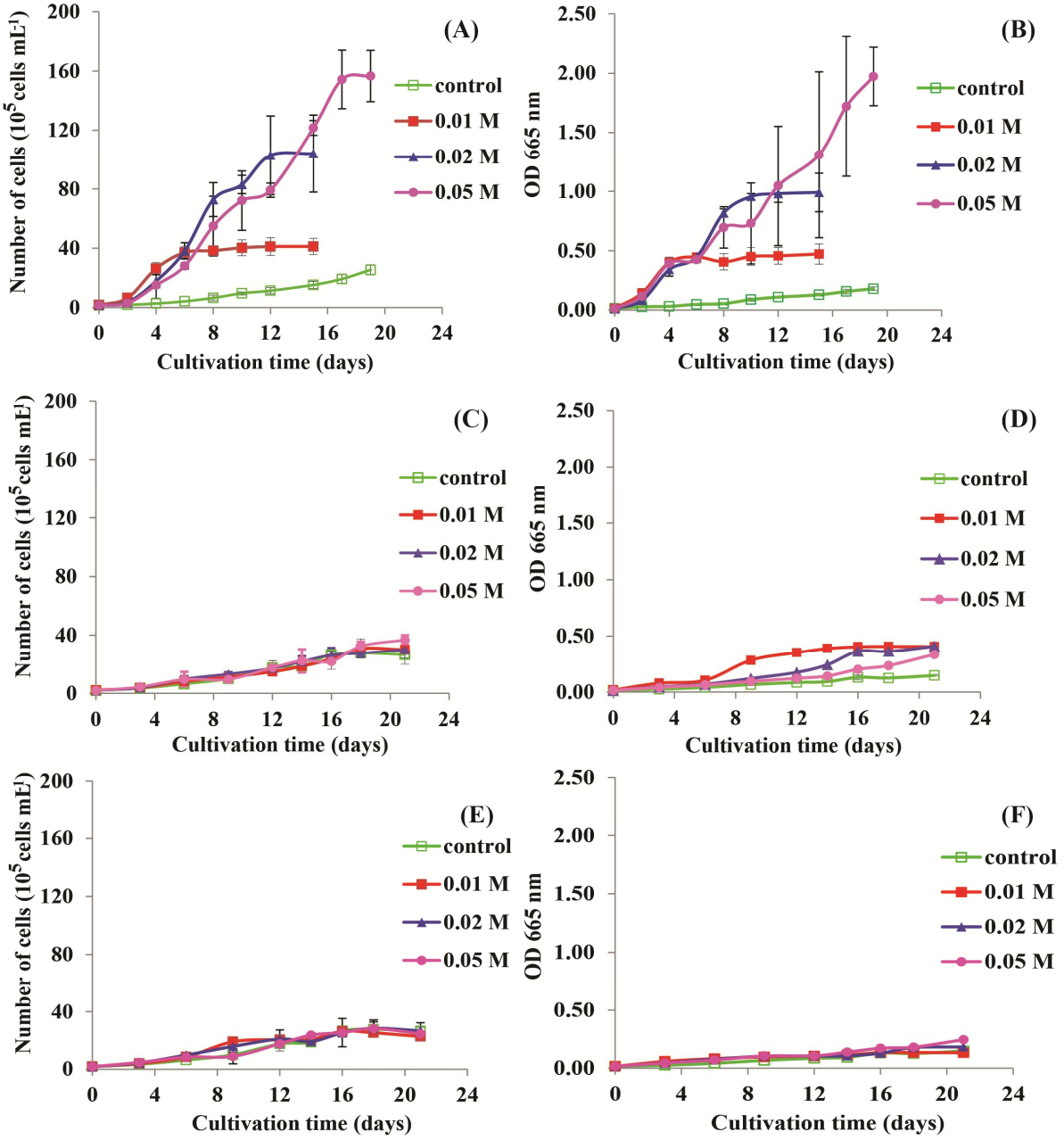

Figure 1. Growth of *Scenedesmus* sp. AARL G022 at 25±2° under continuous illumination in different carbon sources: glucose (A, B); glycerol (C, D); sodium acetate (E, F). Data represent mean ± SD of three replicates.

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

Table 1. Biomass and crude lipid yield from *Scenedesmus* sp. AARL G022 under continuous illumination at 25±2°

Carbon source	Concentration	Biomass ($g.L^{-1}$)	Crude lipid	
			($mg.L^{-1}$)	(% of biomass)
Glucose	control	0.16±0.02 ^{d}	22.55±6.12 ^{bc}	13.49±3.41 ^{a}
	0.01 M	0.35±0.01 ^{c}	38.61±8.56 ^{b}	11.07±2.18 ^{ab}
	0.02 M	1.07±0.06 ^{b}	74.09±13.57 ^{b}	6.77±3.57 ^{b}
	0.05 M	2.78±0.86 ^{a}	233.68±35.34 ^{a}	8.43±1.56 ^{ab}
Glycerol	control	0.12±0.02 ^{d}	17.58±0.98 ^{d}	14.49±2.12 ^{a}
	0.01 M	0.26±0.06 ^{c}	42.10±9.42 ^{c}	16.11±1.61 ^{a}
	0.02 M	0.38±0.09 ^{b}	62.74±14.64 ^{b}	17.06±0.89 ^{a}
	0.05 M	0.47±0.21 ^{a}	67.90±28.26 ^{a}	14.52±0.02 ^{a}
Sodium acetate	control	0.12±0.02 ^{a}	17.58±0.98 ^{b}	14.49±2.12 ^{a}
	0.01 M	0.25 ±0.11 ^{ab}	33.39±10.95 ^{a}	13.89±1.51 ^{a}
	0.02 M	0.25± 0.05 ^{ab}	31.69±6.27 ^{a}	12.68±0.07 ^{a}
	0.05 M	0.30±0.06 ^{a}	35.73±0.80 ^{ac}	12.08 ±1.97 ^{a}

Note: Data represent mean ± SD of three replicates. Different letters indicate significant differences among different concentrations of each carbon source using ANOVA and post hoc Duncan's multiple-range test ($p < 0.05$).

In mixotrophic cultivation, especially with glucose and glycerol supplements, the microalgae could obtain energy from both photosynthesis and oxidation of organic carbon compounds. Thus, they could utilise some part of the energy for cell division, but the excess energy was stored in the form of lipid granules, which could be obviously noticed under a differential microscope. This was also reflected in the morphology, as it resulted in the cells becoming swollen (Figure 2). In addition, some coenobic colonies were observed to be separated into single cells, resulting in non-significant different colony concentrations during the treatment. Moreover, the cells had become enlarged. The cell colour was also observed to change from green to yellow, and the cells were found to be full of lipid vesicles. The lipid-soluble compounds from the autotrophic cells appeared blackish green, what with chlorophyll and carotenoid as the major components, whereas those from the mixotrophic cells appeared light yellow, which was mainly because of the lipid compounds [18]. Therefore, the spectrophotometrical measurement at 665 nm, which corresponds to the chlorophyll concentration in cells, may not be completely correlated to the true algal biomass in mixotrophic culture. A more accurate assessment of growth in the algal mixotrophic cultivation should be performed by dry biomass measurement.

For the sodium acetate supplement, the values of cell concentration, OD_{665}, biomass and lipid content were not significantly different from those under the photoautotrophic condition (Figures 1E, 1F and Table 1). It is true that acetate can be used as a carbon source via acetyl CoA, which can then be changed to pyruvate and further oxidised in the metabolic pathway. However, the result obtained was found to be opposed to that of previous studies which found that sodium acetate could promote both growth and lipid accumulation in many microalgae such as *Phaeodactylum tricornutum* [17], *Brachiomonas submarina* [19] and *Chlorella vulgaris* [11], while *Scenedesmus* sp. AARL G022 seemed unaffected by sodium acetate supplement.

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

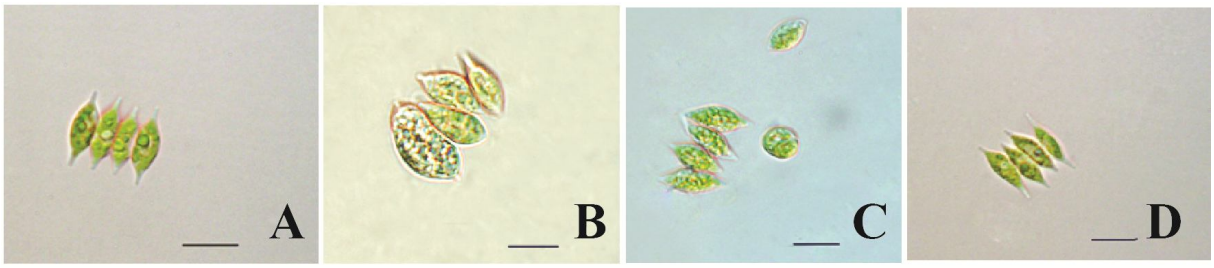

Figure 2. Morphological change of the *Scenedesmus* sp. AARL G022 colony in
A) Photoautotrophic cultivation
B) Mixotrophic cultivation with 0.05 M glucose
C) Mixotrophic cultivation with 0.05 M glycerol
D) Mixotrophic cultivation with 0.05 M sodium acetate

Glucose is the product obtained from the photosynthesis of microalgae; thus, *Scenedesmus* sp. AARL G022 uses glucose directly as an energy source for cell division, rather than stores it in the form of lipid, as evidenced from the supplementation of glucose, which reduced lipid accumulation from 13.49% in the photoautotrophic condition to 8.43% at the 0.05M concentration of glucose supplement. However, the total lipid content was found to increase up to 233.68 $mg.L^{-1}$, which was approximately 10 times that of the control due to the increase in biomass. This is different from that in the case of glycerol supplement, which seems to enhance lipid content and lipid accumulation compared to glucose. Even when the glycerol supplement promoted the algal biomass to become 3-4 times higher than that of the control, its lipid accumulation was not reduced compared to control and this led to a high lipid content in the biomass. This result corresponds to the findings that glycerol can promote the growth of microalgae [20]. The algae, *Schizochytrium limacinum* [21] and *Scenedesmus* sp. [22], were also reported to be capable of glycerol fermentation. In addition, glycerol was also found to enhance fatty acid production in the diatom *Phaeodactylum tricornutum* UTEX-640 [17]. Triglycerides are the main constituents of lipid in algae and it is most probable that glycerol is an intermediate in triglyceride synthesis in algal metabolism. In this study it is clear that glucose supplement promotes cell growth and glycerol supplement enhances lipid accumulation. It would be interesting, therefore, to study the combined effect of glucose-glycerol supplement on both algal growth and lipid content.

Effect of Light-Dark Period

Previous studies have shown that photo-period affects microalgal lipid and biomass production [9, 23]. From our study of the cultures of *Scenedesmus* sp. AARL G022 under photoautotrophic, chemoheterotrophic and mixotrophic conditions, it was found that the alga under mixotrophic condition with a light-dark cycle of 24:0 hr produced the highest number of cells (Figure 3), while the highest biomass was obtained under 24:0 hr and 16:8 hr light-dark cycles (Table 2). However, the highest crude lipid was obtained under 16:8-hr light-dark cycle. Apparently this is because in the light condition (24:0-hr cycle), microalgae perform photoreduction which absorbs energy from light and stores it in energy-carrying molecules such as ATP and NADPH. These energy-pool molecules can be used for the synthesis of biomolecules that promote the growth of the microalgae [9]. Under 16:8-hr cycle, the alga has a dark period in which light-independent reactions can be performed via the Calvin cycle which also operates during the dark phase of photosynthesis.

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

These chemical reactions convert carbon dioxide and other compounds into glucose by using ATP and NADPH from the photoreduction. Microalgae can oxidise supplemented glucose for energy and then store some excess energy in the form of lipids.

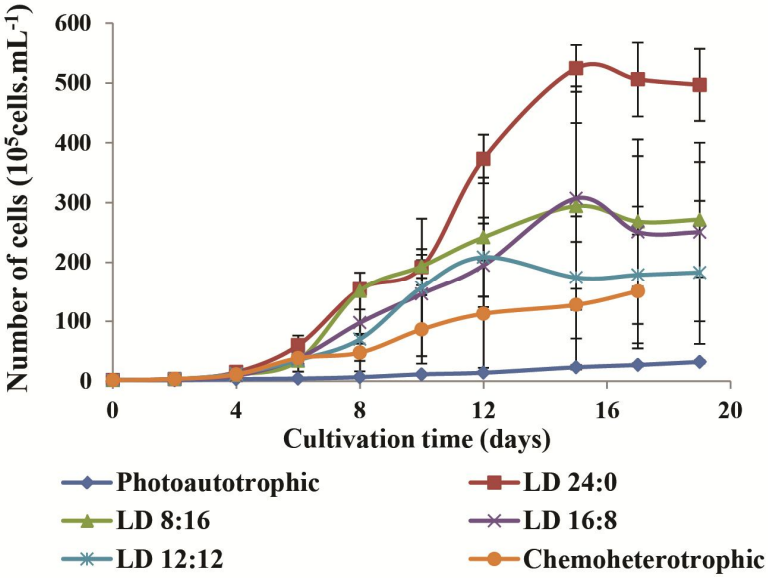

Figure 3. Growth of *Scenedesmus* sp. AARL G022 in the different light-dark cycle (LD) compared with photoautotrophic and chemoheterotrophic cultivation with 0.05M glucose

Table 2. Biomass and crude lipid yield from *Scenedesmus* sp. AARL G022 under different trophic conditions with 0.05M glucose of at 25±2°

	Biomass (g.L^{-1})	**Crude lipid**	
		(mg.L^{-1})	**(% of biomass)**
Photoautotrophic	0.14±0.01 ^{c}	22.57±0.84 ^{d}	15.98±2.30 ^{a}
Chemoheterotrophic	1.94±0.87 ^{b}	158.04±10.89 ^{c}	9.22±4.72 ^{b}
Mixotrophic			
LD 8:16	2.12±0.52 ^{b}	185.00±91.92 ^{c}	8.449 ±2.27 ^{b}
LD 12:12	1.56±0.57 ^{b}	129.16±58.43 ^{c}	8.426 ±2.29 ^{b}
LD 16:8	3.56±0.11 ^{a}	396.35±11.60 ^{a}	11.155±0.13 ^{b}
LD 24:0	4.04±0.36 ^{a}	297.29±51.20 ^{b}	7.471±1.84 ^{b}

Note: Data represent mean ± SD of three replicates. Different letters in the same column indicate significant differences between groups using ANOVA and Duncan's multiple-range test ($p < 0.05$).

Although *Scenedesmus* sp. AARL G022 under mixotrophic cultivation with 0.05M glucose had only a moderate lipid content, its biomass yield was boosted substantially, resulting in high lipid productivity at 396.35 mg.L^{-1} (Table 2). This is comparable with values for species such as *Phaeodactylum tricornutum* (biomass 1.16 g.L^{-1}, 29% lipid content and 336.4 mg.L^{-1} lipid productivity) [17], but lower than values for species such as *Chlorella vulgaris* (biomass 4.8 g.L^{-1}, 13% lipid content and 624 mg.L^{-1} lipid productivity) [11]. In previous studies, it was found that other than the glucose supplement, stress condition and change of nitrogen source could also enhance the productivity. Under a nitrogen-limited condition, *Scenedesmus* sp. 11-1 showed the highest biomass yield of up to 3.88 g.L^{-1} with a lipid content of 41.1% [8], while values of the

specific growth rate and total lipid content of *Scenedesmus dimorphus* and *S. quadricauda* increased when the nitrogen source was changed from nitrate to urea [7]. Thus, a change in the nitrogen source and concentration in a mixotrophic culture may be worth further investigation.

CONCLUSIONS

The optimised condition for culturing *Scenedesmus* sp. AARL G022, a promising organism for bio-oil production, is to cultivate it under a mixotrophic condition with a light-dark cycle of 16:8 hr using 0.05M of glucose supplement. However, glucose is an expensive carbon source so the algal biomass and lipid product are still expensive to be used as raw materials for bio-oil production. Further study should focus on decreasing the media cost by using low-cost nitrogen, phosphorus and organic carbon sources.

ACKNOWLEDGEMENTS

This work was supported by the National Research Council of Thailand (NRCT) under the management of Institute for Science and Technology Research and Development, Chiang Mai University. It was also partially financed by the Energy Policy and Planning Office, Ministry of Energy, Thailand. We thank the Department of Biology, Faculty of Science, Chiang Mai University and the Graduate School for their support.

REFERENCES

1. Y. Chisti, "Biodiesel from microalgae", *Biotechnol. Adv.*, **2007**, *25*, 294-306.
2. X. Li, H. Xu and Q. Wu, "Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors", *Biotechnol. Bioeng.*, **2007**, *98*, 764-771.
3. X. Miao and Q. Wu, "Biodiesel production from heterotrophic microalgal oil", *Bioresour. Technol.*, **2006**, *97*, 841-846.
4. C.L. Peterson, M. Feldman, R. Korus and D.L. Auld, "Batch type transesterification process for winter rape oil", *Appl. Eng. Agr.*, **1991,** *7*, 711-716.
5. Y. Zhang, M. A. Dube, D. D. McLean and M. Kates, "Biodiesel production from waste cooking oil: 1. Process design and technological assessment", *Bioresour. Technol.*, **2003,** *89*, 1-16.
6. A. Bhatnagar, S. Chinnasamy, M. Singh and K.C. Das, "Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters", *Appl. Energ.*, **2011,** *88*, 3425-3431.
7. R. C. D. Goswami and M. C. Kalita, "*Scenedesmus dimorphus* and *Scenedesmus quadricauda*: two potent indigenous microalgae strains for biomass production and CO_2 mitigation—A study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source", *J. Algal Biomass Utln.*, **2011**, *2*, 42-49.
8. J. Liu, C. Yuan, G. Hu and F. Li, "Effects of light intensity on the growth and lipid accumulation of microalga *Scenedesmus* sp. 11-1 under nitrogen limitation", *Appl. Biochem. Biotechnol.*, **2012**, *166*, 2127-2137.
9. J.-V. Pérez-Pazos and P. Fernández-Izquierdo, "Synthesis of neutral lipids in *Chlorella* sp. under different light and carbonate conditions", *C.T.F. Cienc. Tecnol. Futuro*, **2011**, *4*, 47-58.

Maejo Int. J. Sci. Technol. **2014**, *8*(02), 198-206

10. T. M. Mata, A. A. Martins and N. S. Caetano, "Microalgae for biodiesel production and other applications: A review", *Renew. Sustain. Energy Rev.*, **2010**, *14*, 217-232.
11. T. Heredia-Arroyo, W. Wei, R. Ruan and B. Hu, "Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials", *Biomass Bioenergy*, **2011**, *35*, 2245-2253.
12. M. C. Cerón García, A. S. Mirón, J. M. F. Sevilla, E. M. Grima and F. G. Camacho, "Mixotrophic growth of the microalga *Phaeodactylum tricornutum:* Influence of different nitrogen and organic carbon sources on productivity and biomass composition", *Process Biochem.*, **2005**, *40,* 297-305.
13. K. Janta, J. Pekkoh, S. Tongsiri, C. Pumas and Y. Peerapornpisal, "Selection of some native microalgal strains for possibility of bio-oil production in Thailand", *Chiang Mai J. Sci.*, **2013**, *40,* 593-602.
14. J. R. Stein, "Handbook of Phycological Methods: Culture Methods and Growth Measurements", Cambridge University Press, Cambridge, **1979**.
15. E. G. Bligh and W. J. Dyer, "A rapid method of total lipid extraction and purification", *Can. J. Biochem. Physiol.*, **1959**, *37*, 911-917.
16. P. F. Ip, K. H. Wong and F. Chen, "Enhanced production of astaxanthin by the green microalga *Chlorella zofingiensis* in mixotrophic culture", *Process Biochem.*, **2004**, *39*, 1761-1766.
17. H. Wang, R. Fu and G. Pei, "A study on lipid production of the mixotrophic microalgae *Phaeodactylum tricornutum* on various carbon sources", *Afr. J. Microbiol. Res.,* **2012**, *6,* 1041-1047.
18. Q. Y. Wu, S. Yin, G. Y. Sheng and J. M. Fu, "A comparative study of gases generated from stimulant thermal degradation of autotrophic and heterotrophic *Chlorella*", *Prog. Nat. Sci.*, **1992**, *2*, 311-318 (in Chinese).
19. X. Yang, X. Zeng, Y. Ji and Q. Liu, "Effects of sodium nitrate and sodium acetate concentrations on the growth and fatty acid composition of *Brachiomonas submarina*", *J. Ocean Univ. Qingdao*, **2003**, *2*, 75-78.
20. J. Y. Cheng and N. J. Antia, "Enhancement by glycerol of phototrophic growth of marine planktonic algae and its significance to the ecology of glycerol pollution", *J. Fish. Res. Board Can.*, **1970**, *27*, 335-346.
21. D. J. Pyle, R. A. Garcia and Z. Wen, "Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: Effects of impurities on DHA production and algal biomass composition", *J. Agric. Food Chem.*, **2008,** *56,* 3933-3939.
22. H. W. Yen, C. H. Sun and T. W. Ma, "The Comparison of lutein production by *Scenesdesmus* sp. in the autotrophic and the mixotrophic cultivation", *Appl. Biochem. Biotechnol.*, **2011**, *164*, 353-361.
23. E. Jacob-Lopes, C. H. G. Scoparo, L. M. C. F. Lacerda and T. T. Franco, "Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors" *Chem. Eng. Process. Process Intensif.*, **2009**, *48*, 306-310.