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A Selection of Nitrogen Source for Biomass and Lipid Production of *Scenedesmus dimorphus* Microalgae.

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ABSTRACT

Algae are a group of photosynthetic organisms found in many diverse aquatic environments around the world with promising prospect for food to biofuel. In this study, *Scenedesmus dimorphus* species microalgae were cultured in batch cultures to assess the best nitrogen source for lipid production. *S. dimorphus* were cultured in $NaNO_3$, $(NH_4)_2HPO_4$ and urea as nitrogen source respectively and incubated at ± 30 ^{0}C under 50 µmol $m^{-2}s^{-1}$ light intensity with 14:10 h light and dark cycle, aeration was conducted at constant rate of natural air. Growth response was analyzed by measuring optical density (OD) converted to Dry Cell Weight (DCW) and measured periodically. Lipid content were determined by modificated method by Bligh and Dyer. The maximum biomass during cultivation (1.006 ± 0.022 g/ L) was obtained in urea but the highest lipid content of the species (12.98 ± 1.49 %) obtained in sodium nitrate. Fatty acids profile rich in palmitic and oleic acid. The results indicated potential high scale cultivation in urea.

Keywords: biomass, lipid productivity, biodiesel, urea, *Scenedesmus dimorphus*

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INTRODUCTION

Algae are a group of photosynthetic organisms found in many diverse aquatic environments around the world. Compare to higher plant, unicellular structure of microalgae increasing cell surface for capturing light lead to high efficient energy convertion and substrates uptake [1]. The main constituent of microalgae cells are lipids, protein and carbohydrates [2]. Among of these compounds, lipid microalgae has been well studied and shown potential for food to biodiesel. Microalgae can accumulate high amount of lipid (20-50% of dry weight) and with altering environmental factor, lipid content can increase over 60% with lipid class mostly composed of Triacylglyceride (TAG). This is very interesting since can converted into biodiesel via transesterification and known as the third generation biofuel [1, 3]. Lipid component of a few species may contain high valuable Polyunsaturated fatty acids (PUFAs) such as Eicosapentaenoic acid EPA, Docosahexaenoic acid (DHA), Gamma linoleic (GLA) and Arachidonic acid (AA) which can be applied for food suplement and neutraceuticals [4]. DHA from microalgae has been produced commercially but biodiesel from microalgae meet many challenges today dan none is produced commercially [4, 5].

To be commercially, products from microalgae must products must cost competitive to be economics feasible. Selection of algal strains and optimized culture conditions are the key [1]. Optimized culture condition can be achieved by controlling enviromental factors and nutrients [6]. Nutrients composition are very important since can affect to biochemical composition of microalgae and have been used to hyper accumulation of many valuable substances [7]. Nitrogen, phosphor and carbon are primary compounds for algae cell and a central of algae biotechnology. Nitrogen in cell composed about 7–10% of cell dry weight plays important role to support the growth and reproduction [8]. Effect of nitrogen sources on the composition of microalgae has been well studied. Many of them focus on effect of nitrogen depletion and repletion to biochemical components such as lipids, proteins, carbohydrates, and pigments (carotenoids) [6] but less reported for alternative nitrogen sources. Meanwhile, various of synthetic medium developed for microalgae commonly used inorganic substance that are not produced universally and expensive. Purpose of this study was to evaluate growth *S. dimorphus* microalgae under different nitrogen source for lipid production.

MATERIAL AND METHODS

Algal and cultivation conditions

The *S. dimorphus* microalgae was from the collection of Laboratory of Biochemistry Andalas University. The species has been isolated previously from freshwater river in Padang province, Indonesia and was identified based on morfological characterstics. Pure cells of the algae species were achieved by streaking method in modified Bolt`s Basal Medium (BBM) contains 1-2% agar and cultured under continous light. After a week growth, the culture was observed under microscope light with 1000x magnification. Cell colony of the species was then picked up and pre-cultured in BBM medium with working volume 1 L in botlle glass. Cultivation under different nitrogen sources were performed in erlemenyer flask with 500 ml of volume culture and incubated at ± 30 ^{0}C under 50 µmol $m^{-2}s^{-1}$ light intensity, 14:10 h light and dark cycle, and constant rate of natural air as aeration. The experiment wsd conducted in triplicate.

Measurement of cell growth

The growth of microalgae measured periodically by measuring optical density (OD) converted to dry cell weight (DCW) was established by plotting OD 550 nm versus DCW of a series of samples of different biomass concentrations. For DCW determination, 25 mL of the culture samples were filtered through pre-weighted 1.2 µm glass-fiber filters APFC (Millipore) under vacuum and oven dried at 60 ^{o}C for 2 h. Based on regression equation, 1 OD was equivalent to 0.5294 g/L DCW (r^2 = 0.9919).

Lipid extraction

Lipid was extracted by solvent extraction [9] as well as modified [10, 11]. Samples were harvested by gravitational settling followed by centrifugation at 4000 rpm 5 min subsequently freeze drying. 0.1 g freeze dried biomass was suspended into 5 ml dH_2O and distrupted by microwave oven for 5 min. after centrifugation at 3000 rpm for 10 min, pelet was mixed with 6 ml of solvent (chloroform-methanol 1:1) vortex 30 s. 2 ml of

solvent and 2 ml dH_2O were then added and vortex again. After centrifugation 2.000 rpm for 10 min, lipid fraction was transferred into pre-weighed clear vial tube. Re-extraction was performed to remain pelet using 4 mL of solvent. The combined solvent was filtered through Whatman No. 1 filter paper in a funnel subsequently evaporated by oven dried 50 ^{o}C for 10 h. Lipid content was then determined gravimetrically.

Fatty acids measurement by gas chromatography mass spectroscopy (GC-MS)

Fatty acids methyl ester was determined as Lepage & Roy modification methods by Laurens *et. al* [12] with slighly modified. Sample 10 mg of dry biomass was soaked with 0.2 ml chloroform/methanol (1:1) containing 1 mg/ml methyl nonadecanoate as internal standar. 0.3 ml HCl/methanol (5%) was added and incubated at 82 ^{o}C in waterbath for 90 min. After cooling to room temperature, hexane (2 x 1 ml) was added and left exracted 1 hour in room temperature. Hexane layer was then recovered, diluted and saved -20 C before analysis. FAME was injected to Gas Chromatography Mass Spectroscopy (GC/MS) using DB-WAX column. Column oven temperature was 60 ^{o}C (2 min hold) and then initially increased to 150 ^{o}C (2 min hold) at rate of 10 ^{o}C/min and then to 220 ^{o}C (5 min hold) at a rate of 10 ^{o}C/min. The injector temperature was 200 ^{o}C with split ratio 10.0. Fatty acid was identified based on Mass Spectrum fragmentation compared to database. Quantification of fatty acid was performed by comparing percent area relative to internal standar.

RESULTS

Effect of nitrogen sources on growth

In this study, we done tested of nitrogen sources such as sodium nitrate ($NaNO_3$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and urea to the growth response represented as biomass production of the species. $NaNO_3$ was selected because it is a standard nitrogen source most of synthetic mediums while $(NH_4)_2HPO_4$ containing phosphate beside ammonium which can support the growth and urea for availability and inexpensive. Among of nitrogen sources tested, *S. dimorphus* showed better growth in urea while ($(NH_4)_2HPO_4$) and NH_4Cl can only support rather poor growth as shown in Fig 1. Maximum biomass concentration obtained with urea as the nitrogen source was 1.006 ± 0.022 g/L slightly higher than $NaNO_3$ (0.896 ± 0.026 g/L), $(NH_4)_2HPO_4$ (0.7653 ± 0.020 g/L) and NH_4Cl (0.268 ± 0.029 g/L) for 25 days of cultivation respectively.

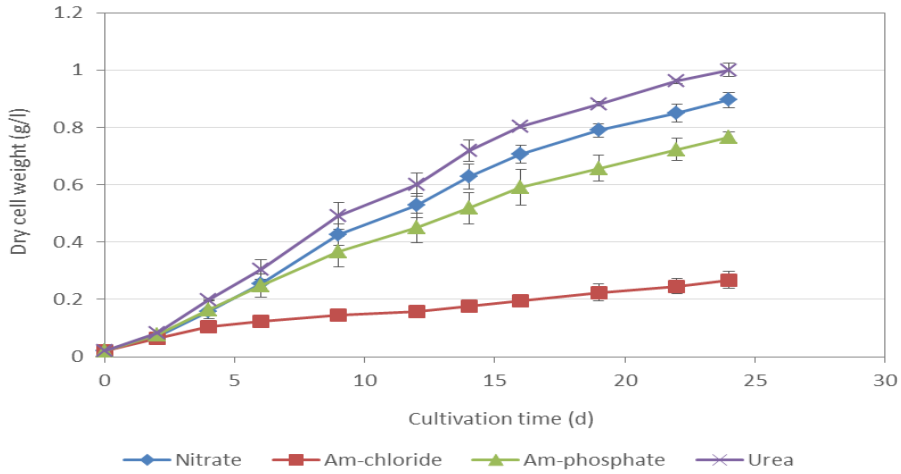

Figure 1: Comparison of growth of *Scenedesmus dimorphus* microalgae cultured in different nitrogen sources in BBM media consisting of 3 mM sodium nitrate, diammonium-hydrogen phosphate or urea respectively. Data represents Mean ± SD (n=3)

Trend pH of nitrogen sources was shown in Fig 2. Among of nitrogen sources tested, urea showed relatively stable in the neutral pH range whereas $(NH_4)_2HPO_4$ and NH4Cl showed a tendency to acidic and $NaNO_3$ has the opposite effect. Trend pH of medium caused by release of H^+ and OH^- of nitrogen species when assimilated by microalgae [13].

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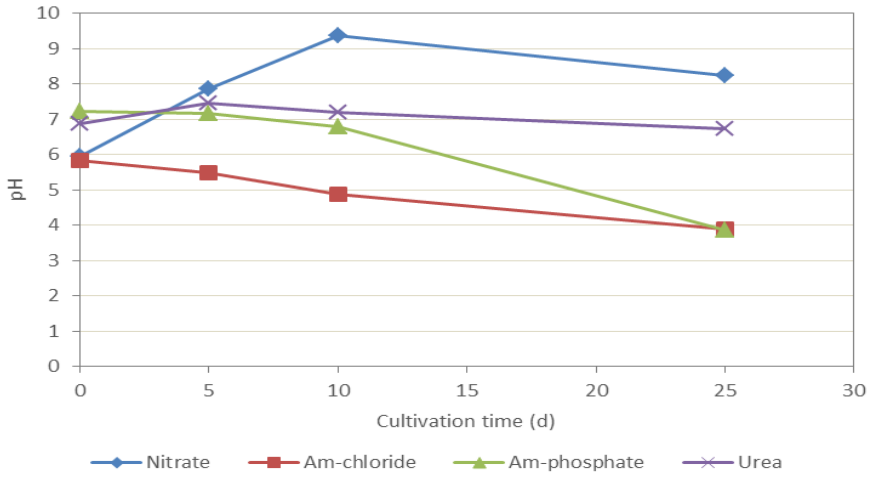

Figure 2: Change of pH during cultivation with different nitrogen sources in BBM media consisting of 3 mM sodium nitrate, diammonium-hydrogen phosphate or urea respectively.

Nitrogen in the ammonium form is faster assimilated by microalgae indeed but easier to be toxic for microalgae. Concentrations above 25 µM frequently reported to be toxic for microalgae [7]. It might lead to poor growth of microalgae in $(NH_4)_2HPO_4$) and NH_4Cl especially in ($(NH_4)_2HPO_4$) even contains phosphate that expected to support growth. In other hand, significant change of nitrogen sources pH during cultivation also might be affect to algae growth. As shown in fig. 1 poor growth of the algae in $(NH_4)_2HPO_4$) and NH_4Cl relevant to tendency of pH each nitrogen sources during cultivation.

Effect of nitrogen sources on lipid production

Biomass harvested subjected to lipid extraction was shown in Fig 3. The highest lipid content obtained from culture containing $NaNO_3$ (12.98 ± 1.49 %). This is not significantly different with urea (12.5 ± 0.07 %) but approximately a half times higher than $(NH_4)_2HPO_4$ (8.43 ± 1.47 %) respectevely. Higher lipid productivity was obtained in urea (5.025 ± 0.14 mg L^{-1} day^{-1}) slightly higher than $NaNO_3$ (4.85 ± 0.0.69 mg L^{-1}) and approximately two times higher than $(NH_4)_2HPO_4$ (2.68 ± 0.30). Better growth response in urea even in low lipid content make lipid productivity of urea was very comparable with $NaNO_3$.

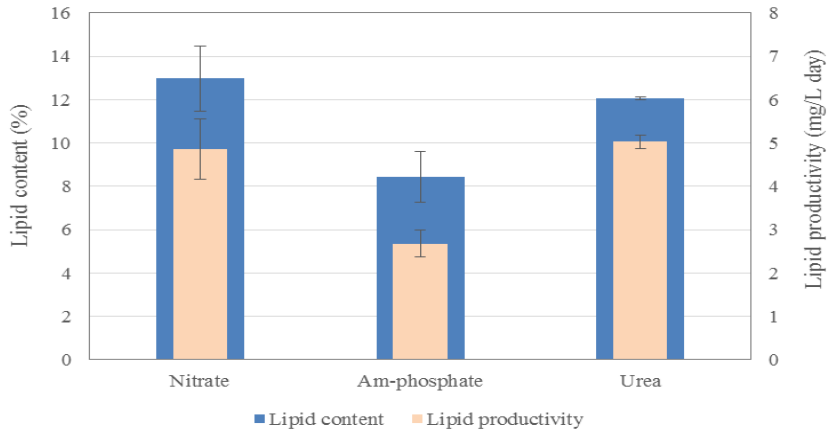

Figure 3: Lipid content and lipid productivity of *Scenedesmus dimorphus* in media consisting of 3 mM sodium nitrate, diammonium-hydrogen phosphate or urea respectively. Data represents Mean ± SD (n=3).

Fatty acids composition

Fatty methyl ester determinations showed Fatty acids composition only consist of Hexadecanoic acid, methyl ester/Palmitic acid (71.14 %) and 9-Octadecenoic acid, methyl ester/oleic acid (28.86 %) as shown in

table. 1 respectively. Fatty acids content in cells were 2.62 % (palmitic acid) and 1.06 % (oleic acid) of dry weight respectively. Compare to other study, there are lack of some fatty acids and might be the effect of cultivation conditions especially application of urea as nitrogen instead of nitrate [14].

Table No 1. Fatty acids content of *S.dimorphus* in urea

	Fatty Acids	Composition (%)	Content (% dw)
C16:0	Hexadecanoic acid, methyl ester (Palmitic acid)	71.14	2.62
C18:1	9-Octadecenoic acid, methyl ester (oleic acid)	28.86	1.06
	Total	100.00	3.68

In additions, several other species are also found growth optimally in urea such as *Phaodactilum tricornutum*, *Synecoccus colbassu* [15] even many other still favorable with nitrate [15-18]. Compare to nitrate, urea is less expensive and widely available. Furthermore, urea has other advantages that stable trend of pH during cultivation make it more friendly to the environment compared to $NaNO_3$. However, further research is needed especially to optimize a variety of environmental factors and other nutrients in order to obtain high lipid productivity of this species in urea but still economics.

CONCLUSION

Biomass production of *S. dimorphus* showed better in urea than other nitrogen sources used in this study. Both lipid content and lipid productivity were not significantly differs between urea and sodium nitrat but lower results observed in $((NH_4)_2HPO_4)$ and NH_4Cl. This results suggest that urea can subtitutes sodium nitrat as nitrogen source in medium for biomass and lipid production of *S. dimorphus* microalgae and potential to be developed to high scale culture.

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