Effect of Different Nitrogen Sources on Growth and Biochemical Composition of Microalgae for Aquaculture Feed

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Abstract

This project isolated microalgae from natural sources and studied the effect of nitrogen sources on growth of microalgae *Monoraphidium* sp. and *Desmodesmus* sp. when cells were grown in BG11 medium. The difference sources of nitrogen have been used in the experiment such as $NaNO_3$ (3.45 mmol/L), NH_4Cl (2.56 mmol/L) and $NaNO_3$: NH_4Cl in equimolar concentration by using medium without nitrogen source as the control. The results shown that the higher growth rate and protein content was found in *Desmodesmus* sp. when using nitrogen source as $NaNO_3$. But higher lipid production was found in *Desmodesmus sp*. when grown cell in medium without nitrogen source, respectively.

Keywords : Nitrogen source, Microalgae, Growth and Biochemical Composition

1.Introduction

Microalgae is microorganism which can be found in freshwater. It has many variety of species and more benefit. Microalgae are rich in the quantity of nutritious compounds as proteins, carbohydrates, lipids, vitamins, pigments, minerals and other nutraceuticals [1]. Essential nutrients for the growth of microalgae including carbon (C), hydrogen (H), oxygen (O), phosphorus (P), Potassium (K), sulfur (S), Magnesium (Mg) and nitrogen (N). The research found nitrogen changes have a major effect on biochemical compounds such as lipid and protein [2]. Nowadays, there are numerous commercial applications of microalgae. For example, microalgae can be used to enhance the nutritional value of food and animal feed owing to their chemical composition, they play a crucial role in aquaculture and they can be incorporated into cosmetics. Moreover, they are cultivated as a source of highly valuable molecules. For example, polyunsaturated fatty acid oils are added to infant formulas and nutritional supplements and pigments are important as natural dyes. There has been an increasing interest in using of green freshwater microalgae as *Desmodesmus* sp. and *Monoraphidium* sp. for producing protein and lipid as aquaculture feed [3]. However, different nitrogen sources or limitation of nitrogen source will affected on chemical composition of microalgae because nitrogen is a major constituent of protein and nucleic acid, which important for transmission of genetic information in organisms [4]. The main of nitrogen sources in water with come from nitrate and ammonia. Therefore, the aim of this project was to study the effect of different nitrogen sources on growth and biochemical composition of two isolated microalgal strain, *Desmodesmus* sp. and *Monoraphidium* sp. for searching the optimum condition to product the aquaculture Feed.

2. Materials and methods

2.1 Microalgae isolation

Water samples were collected from the pool area at Rajamangala University of Technology Thanyaburi (RMUTT). Microalgae were purified by using pick cell technique and observed under compound microscope until get the single cells of each strain. Then cell was transferred into a bottle of 5 ml of BG11 medium to enhance amount of cells before preparation of stock culture in 200 ml of BG11 medium and incubated at 25^{o}C under continuous illumination.

2.2 Method design

Two isolated microalgae strains, *Desmodesmus* sp. and *Monoraphidium* sp. were cultured in 15 L of BG11 medium in 20 L of tank reactor by vary nitrogen sources as $NaNO_3$ (3.45 mmol/L), NH_4Cl (2.56 mmol/L) and $NaNO_3$: NH_4Cl in equimolar concentration using medium without nitrogen source as the control. The cell culture was incubated under same growth condition before collecting cell sample for analysis of growth rate, chlorophyll *a* content, protein and lipid, respectively.

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2.3 Analysis of cell growth and biochemical composition

- Cell counting under light microscope by using haemacytometer in every day of cultivation for determination of cell growth. The experiment had been repeated three times.

- Chlorophyll *a* content was analyzed by taking 20 ml of cell sample from a tank and then filtered by GF/C to remove medium out and then put cells on GF/C into the 90% methanol and boiled at a temperature of 70 ° C for 2 hours to extract chlorophyll *a*. Sample was centrifuged at 4000 rpm for 10 minutes before measuring the absorbance at 653 and 666 nm wavelengths by spectrophotometer.

- Protein analysis was performed by using the standard Kjeldahl method (2005). Cells at 20 days of cultivation were collected and lyophilized by freeze-dryer before dry cells were digested with 80 % (v/v) of sulfuric acid at high temperature for one hour and using $CuSO_4 \cdot 5H_2O$ and K_2SO_4 as a catalyst. At the end of the process nitrogen in inform of $(NH_4)_2SO_4$ was titrated to calculate the amount of protein in the sample.

- Lipid analysis was performed by using Soxhlet's Methods, 2 g of dry sample was extracted and then measured lipid after distillation.

3. Results and Discussions

The Figure 1 shows that *Desmodesmus* sp. grown well in medium containing $NaNO_3$ as nitrogen source at the amount of cells of 99×10^4 (Table 1). This result suggested that nitrate is optimum nitrogen source for supporting cell growth which consistent with the result of Bálint and co-worker [4]. The limiting nitrogen affects the photosynthesis of microalgae cells [4]. As a result, the growth rate and amount of chlorophyll *a* were decreased in condition without nitrogen source (Fig. 1).

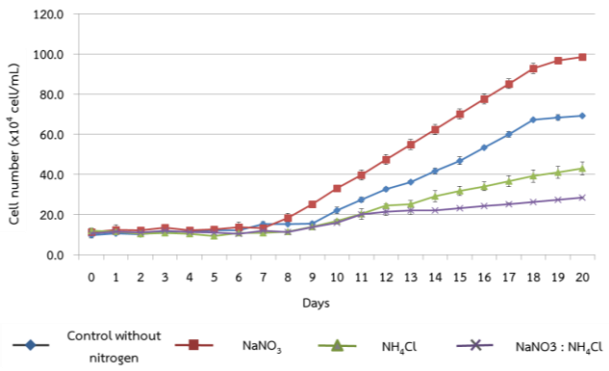

Figure 1 Cell growth of *Desmodesmus* sp. when grown cell in medium containing different nitrogen sources.

The Figure 2 shows that *Monoraphidium* sp. grown well in medium without nitrogen source at the amount of cells of 1800×10^4 as shown in Table 1. This results suggested that *Monoraphidium* sp. could grow in medium without nitrogen source, which correlation result with Ammerman *et al*. [5]. that have been reported that phosphorus is a vital to the growth of microalgae, as well as nitrogen. Although there is no nitrogen in the water.

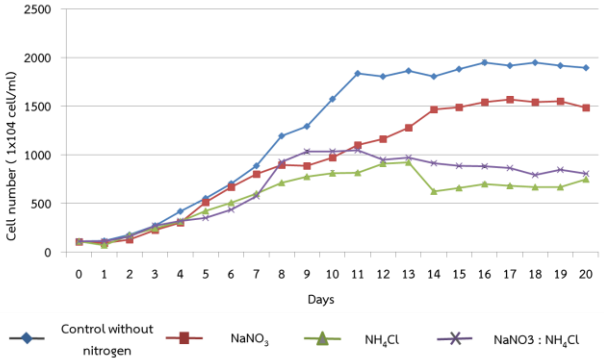

Figure 2 Cell growth of *Monoraphidium* sp. when grown cell in medium containing different nitrogen sources.

The highest chlorophyll *a* content of *Desmodesmus* sp. was found in culture without nitrogen source, the maximum content was 26 mg/g (Fig.3). And the chlorophyll *a* content of *Monoraphidium* sp. was found in culture containing $NaNO_3$, the maximum content was 11 mg/g (Fig. 4). However, *Desmodesmus* sp. show higher chlorophyll *a* content than *Monoraphidium* sp.

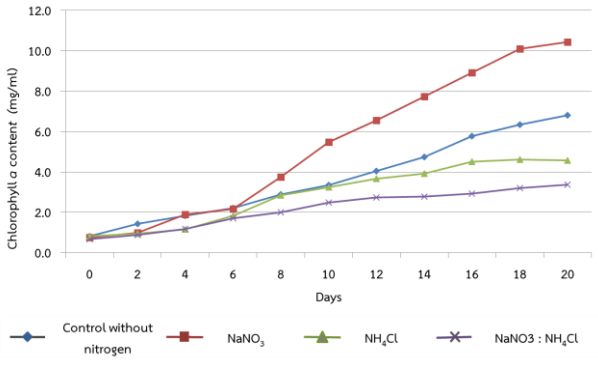

Figure 3 Chlorophyll *a* content of *Desmodesmus* sp. when grown cell in medium containing different nitrogen sources.

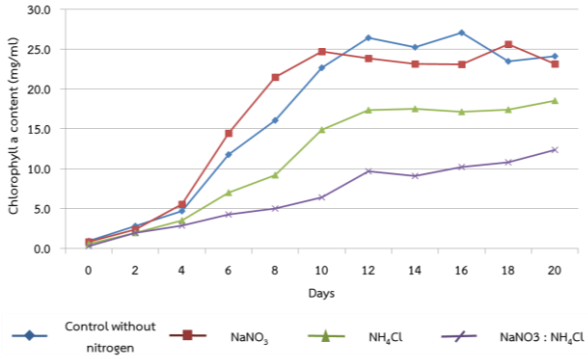

Figure 4 Chlorophyll *a* content of *Monoraphidium* sp. when grown cell in medium containing different nitrogen sources.

The maximum protein content, was 12 %(w/w) dry weight, was found in cell of *Desmodesmus* sp. when grown cell in medium containing $NaNO_3$ (Fig. 5), which consistent with Chen *et al*. [6] that revealed the nitrogen is a major constituent of protein and nucleic acid of microalgae. It's deficiency results in decease of protein production [6]. Interesting, the *Monoraphidium* sp. showed the maximum protein content, was about 40 %(w/w) dry weight, when grown cell in culture containing $NaNO_3$ (Fig. 6).

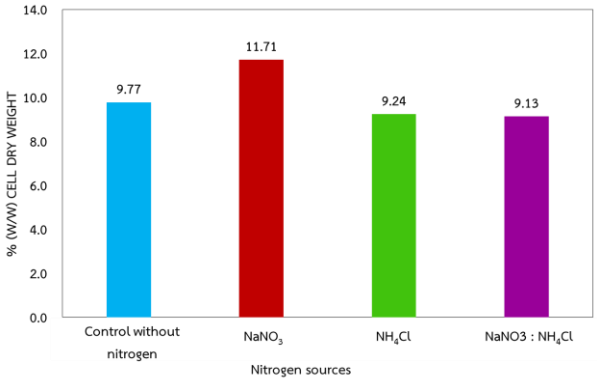

Figure 5 Protein content of *Desmodesmus* sp. when grown cell in medium containing different nitrogen sources.

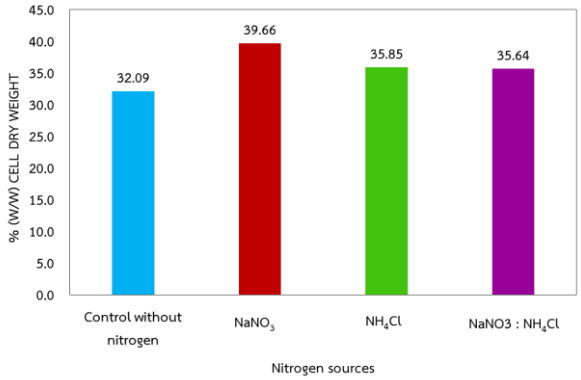

Figure 6 Protein content of *Monoraphidium* sp. when grown cell in medium containing different nitrogen sources.

In addition, Lipid production of *Desmodesmus* sp. showed the content (about 7 - 15 %(w/w) dry weight) in all conditions (Fig.7). But lipid production of *Monoraphidium* sp. showed lower content (3 – 7 %(w/w) dry weight) than *Desmodesmus* sp. (Fig. 8). Then, the results revealed that the highest lipid content was found in *Desmodesmus* sp. when cells were cultured in medium without nitrogen source (Table 1).

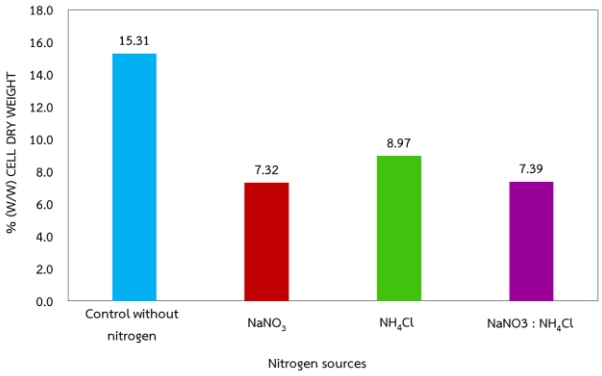

Figure 7 Lipid content *Desmodesmus* sp. when grown cell in medium containing different nitrogen sources.

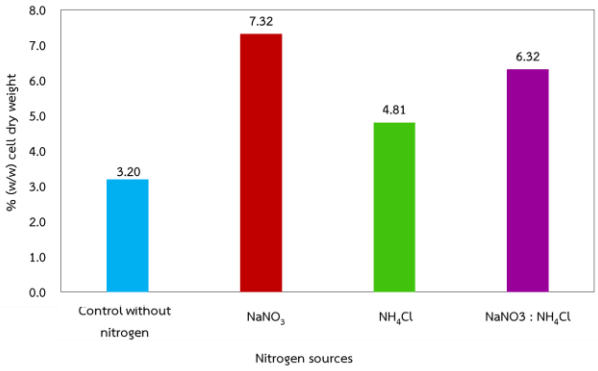

Figure 8 Lipid content *Monoraphidium* sp. when grown cell in medium containing different nitrogen sources.

Table 1 Comparison of growth rate, chlorophyll *a*, protein and lipid contents of microalgae when cells were grown under different nitrogen sources for 20 days.

Value Analysis	*Desmodesmus* sp.				*Monoraphidium* sp.			
Nitrogen sources	No nitrogen added	$NaNO_3$	NH_4Cl	MIX	No nitrogen added	$NaNO_3$	NH_4Cl	MIX
Number of cell ($1x10^4$ cell/ml)	69.30	98.55	43.00	28.35	1892.42	1485.25	747.00	803.83
Chlorophyll *a* content (mg/ml)	6.82	10.44	4.56	3.38	24.11	23.18	18.53	12.38
Protein (%w/w of cell dry weight)	9.77	11.71	9.24	9.13	32.09	39.66	35.85	35.64
Lipid (%w/w of cell dry weight)	15.31	7.32	8.97	7.39	3.20	7.32	4.81	6.32

4. Conclusion

The results showed that nitrogen source has a major effect of growth and protein content of *Desmodesmus* sp. and *Monoraphidium* sp. The nitrate was optimum source for supporting microalgae growth and chlorophyll *a* content. Therefore, nitrate is a good source for enhance biomass of microalgae suitable for aquaculture feed. However, the lipid production will be increased when microalgae were grown in medium without nitrogen source. It might be cause of microalgae have to accumulated lipid for spare energy when cells stay under nitrogen starvation.

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