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The preliminary study of lipid production of *Nostoc* sp. from Bueng Boraphet, Nakhon Sawan Province

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Abstract

Our study was focused on optimum physical and nutritional conditions in lipid accumulation of microalgae (*Nostoc* sp.) from Bueng Boraphet using BG-11 as cultured media. Results showed that the optimum temperature and acid-base value were at 30 °C and pH 7.0, respectively under autotrophic condition. *Nostoc* sp. was able to properly grow and gave 0.517 g/L dry cell weight and 4.16% lipid content. The optimum nutrition for lipid accumulation by *Nostoc* sp. was then examined. These conditions were found to be 0.15% potassium nitrate (KNO_3), 10% glucose and 0.004% dipotassium phosphate (K_2HPO_4) at 30 °C, pH 7.0 under mixotrophic condition. *Nostoc* sp. showed the highest dry cell weight and lipid content at 1.314 g/L and 4.48 percent respectively. The trend of lipid accumulation was depend on the growth rate of *Nostoc* sp.

Keywords : *Bueng Boraphet, Microalgae, Lipid production*

1. Introduction

Nowadays, increment of population and economic development cause the high demand of worldwide energy consumptions. This problem creates the condition of declining of non-renewable energy resources. Biodiesel is regarded as alternative energy which is gaining an interest from consumer. Since, biodiesel could be produced using organic oils from plant, algae and animal fats. Especially, algae which is a potential source of renewable fuel. Algae is an autotrophic organism. It can produce oil through photosynthesis using sunlight and then converted oil into fatty acid methyl esters through the process of tranesterification (1). A unicellular algae is regarded as microalgae. Cyanobacteria or blue green alga is considered as one type of microalgae which live in natural habitat such as lake, pond, spring, stream and river. This algal type is widely found in open pond and almost has a high growth rate. Previous research reported that the high population of many microalgae provided the high lipid contents (2). On the contrary, some microalgae species is exposed that a different result. The high lipid productivity was detected in extreme condition which algae demonstrated its slow growth rate (3). The high yield of biodiesel should be obtained from high lipid contents. This fact encourage worldwide researcher to observe an optimal conditions for high lipid accumulation in microalgae.

2. Materials and Methods

2.1 The source of microalgae

Microalgae *Nostoc* sp. was isolated from Bueng Boraphet, or Boraphet marsh that located in Nakhon Sawan province. BG-11 medium was used to culture and stored this microalgae.

2.2 Physical factors on lipid production

2.2.1 Influence of light

The algal *Nostoc* sp. was cultured in BG-11 broth at 30 degree celcious with continuous shaking at 100 rpm for 14 days under illuminance (autotrophic condition) and non-illuminance (heterotrophic condition).

2.2.2 pH and temperature optimization

The effect of pH and temperature on lipid accumulation was assessed by variation these physical factors in BG-11 broth. The media were varied pH as 6.5, 7.0 and 7.5 and incubated at 30 degree celcious with continuous shaking at 100 rpm for 14 days under autotrophic condition. Cultured media were adjusted to optimal pH and incubated at 25, 30, 37 degree celcious with continuous shaking at 100 rpm for 14 days under autotrophic condition.

2.3 Chemical factors on lipid production

2.3.1 Optimization of nitrogen, carbon and phosphorus sources

Several nitrogen sources were assessed with using yeast extract, urea, potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$; control) at concentration 0.15% (w/v). Cultured media were incubated at 30 degree celcious with continuous shaking at 100 rpm for 14 days under autotrophic condition

Optimal nitrogen source was used instead of $NaNO_3$ and supplemented with various concentration of glucose at 1, 5 and 10% (w/v) in BG-11 broth. Cultured media were incubated at 30 degree celcious with continuous shaking at 100 rpm for 14 days under mixotrophic condition.

BG-11 broth was prepared by using optimal nitrogen source and optimal g lucose concentration. Then, Dipotassium hydrogen phosphate (K_2HPO_4) was finally tested by varying concentration at 0.002, 0.004 and 0.006% (w/v). Cultured media were incubated at 30 degree celcious with continuous shaking at 100 rpm for 14 days under mixotrophic condition

2.4 lipid measurement assay

Culture broth will be collected every 2 days to measure dry cell weight and lipid content in algal cell. Dry cell (1 gram) of microalgae was extracted the total lipid by modification method of Bligh and Dyer (4). Dry microalgal cell was ground with solvent mixture of Chloroform, methanol and distilled water at ratio 1 : 2: 0.8 for 2 minutes and then stirred for 4 hours at room temperature. Chloroform (100 ml) was then added and mixed for 30 seconds. Then, distilled water (100 ml) was added and continuously shaked for 30 seconds. The mixture was brought to centrifuge at 4,150 rpm for 10 minutes and allowed it to separate into three layers. Bottom layer of chloroform was collected and discarded top layer of methanol. The remaining layer of biomass was extracted twice more. The chloroform extract was kept at 4 degree celcious overnight. The volume of extract was then measured in graduated cylinder. Total lipid in chloroform extract was checked gravimetrically by heating an aliquot of the extract in preweighed aluminum dish. The weight of pooled chloroform extract was calculated follow this equation.

$$\text{Total lipid (\%)} = \frac{A \times B}{C}$$

A = volume of chloroform extract (ml)
B = total lipid concentration in extract (mg/ml)
C = mass of biomass extracted (mg)

3. Results

3.1 Physical factors on lipid production

Under autotrophic condition, *Nostoc* sp. was able to properly grow and demonstrated the lipid content higher than heterotrophic condition. Under autotrophic condition, dry cell weight and lipid concentration were 0.411 ± 0.047 g/L (Table 1) and 3.84% (Figure 1A), respectively. The effect of temperature and pH on cell growth were then experimented. The highest cell growth and lipid content were obtained from pH 7.0 and 30 degree celcious. These conditions were found to be an optimal condition for both the cell growth and lipid production. *Nostoc* sp. was able to accumulate lipid reached to 4.16% (Figure. 1C).

3.2 Chemical factors on lipid production

Nostoc sp. preferred potassium nitrate (KNO_3) to other nitrogen sources. The lipid production was 4.7 folds higher when microalgae was cultivated on potassium nitrate than on yeast extract (Figure 2A). Under mixotrophic condition, lipid production varied directly with the concentration of glucose. It was found that, at 10% (w/v) of glucose gave the highest of

Table 1. Effect of physical parameters on growth of *Nostoc* sp. (g/L)

Parameters \ Days	2nd	4th	6th	8th	10th	12th	14th
Non-Illumination	0.010 ± 0.003	0.191 ± 0.017	0.197 ± 0.017	0.231 ± 0.009	0.238 ± 0.009	0.242 ± 0.008	0.276 ± 0.005
Illumination	0.041 ± 0.037	0.225 ± 0.009	0.262 ± 0.024	0.279 ± 0.019	0.317 ± 0.057	0.374 ± 0.016	0.411 ± 0.047
pH 6.5 (at 30 °C)	0.053 ± 0.006	0.139 ± 0.025	0.200 ± 0.003	0.243 ± 0.059	0.301 ± 0.036	0.340 ± 0.055	0.407 ± 0.059
pH 7.0 (at 30 °C)	0.091 ± 0.007	0.180 ± 0.025	0.231 ± 0.039	0.331 ± 0.068	0.380 ± 0.036	0.421 ± 0.029	0.475 ± 0.032
pH 7.5 (at 30 °C)	0.084 ± 0.004	0.155 ± 0.042	0.213 ± 0.046	0.280 ± 0.078	0.334 ± 0.003	0.408 ± 0.023	0.430 ± 0.034
25 °C (at pH 7.0)	0.213 ± 0.003	0.242 ± 0.011	0.305 ± 0.011	0.328 ± 0.004	0.340 ± 0.002	0.424 ± 0.081	0.470 ± 0.021
30 °C (at pH 7.0)	0.298 ± 0.005	0.310 ± 0.001	0.319 ± 0.008	0.335 ± 0.005	0.383 ± 0.030	0.484 ± 0.069	0.517 ± 0.025
35 °C (at pH 7.0)	0.207 ± 0.004	0.230 ± 0.005	0.263 ± 0.008	0.319 ± 0.003	0.344 ± 0.014	0.386 ± 0.006	0.479 ± 0.015

Table 2. Effect of chemical parameters on growth of *Nostoc* sp. (g/L)

Parameters / Days	2nd	4th	6th	8th	10th	12th	14th
Yeast extract	0.037 ± 0.006	0.157 ± 0.008	0.182 ± 0.009	0.205 ± 0.022	0.222 ± 0.0026	0.274 ± 0.054	0.326 ± 0.006
Urea	0.044 ± 0.014	0.190 ± 0.018	0.223 ± 0.042	0.255 ± 0.026	0.284 ± 0.010	0.339 ± 0.023	0.397 ± 0.006
KNO_3	0.118 ± 0.006	0.250 ± 0.043	0.279 ± 0.024	0.294 ± 0.009	0.499 ± 0.179	0.679 ± 0.094	1.314 ± 0.020
Control	0.069 ± 0.013	0.228 ± 0.008	0.261 ± 0.033	0.287 ± 0.005	0.339 ± 0.059	0.456 ± 0.050	0.565 ± 0.109
1% glucose	0.239 ± 0.021	0.310 ± 0.017	0.502 ± 0.035	0.543 ± 0.015	0.555 ± 0.004	0.604 ± 0.004	0.610 ± 0.001
5% glucose	0.798 ± 0.004	0.884 ± 0.016	0.936 ± 0.020	0.947 ± 0006	0.976 ± 0.006	1.068 ± 0.048	1.112 ± 0.004
10%glucose	1.507 ± 0.013	1.533 ± 0.037	1.578 ± 0.012	1.597 ± 0.004	1.646 ± 0.052	1.704 ± 0.035	1.737 ± 0.036
0.002%K_2HPO_4	0.015 ± 0.002	0.032 ± 0.004	0.102 ± 0.002	0.251 ± 0.002	0.339 ± 0.005	0.420 ± 0.003	0.423 ± 0.001
0.004% K_2HPO_4	0.080 ± 0.006	0.128 ± 0.005	0.222 ± 0.002	0.344 ± 0.011	0.442 ± 0.006	0.513 ± 0.004	0.569 ± 0.002
0.006% K_2HPO_4	0.055 ± 0.004	0.084 ± 0.006	0.217 ± 0.009	0.298 ± 0007	0.426 ± 0.005	0.476 ± 0.008	0.471 ± 0.002

cell dry weight of microalgae (Table 2). However, lipid production was slightly reduced when cultured in mixotrophic condition. The effects of dipotassium hydrogen phosphate (K_2HPO_4) concentration on lipid production were then studied under mixotrophic condition. The optimal concentration of phosphorus was 0.004% (w/v) which demonstrated the highest cell growth and lipid content. *Nostoc* sp. could accumulate lipid in their cells reached to 4.48% (Figure 2C). The results of all parameters in this research suggested that lipid accumulation was directly varied with dry cell weight of microalgae.

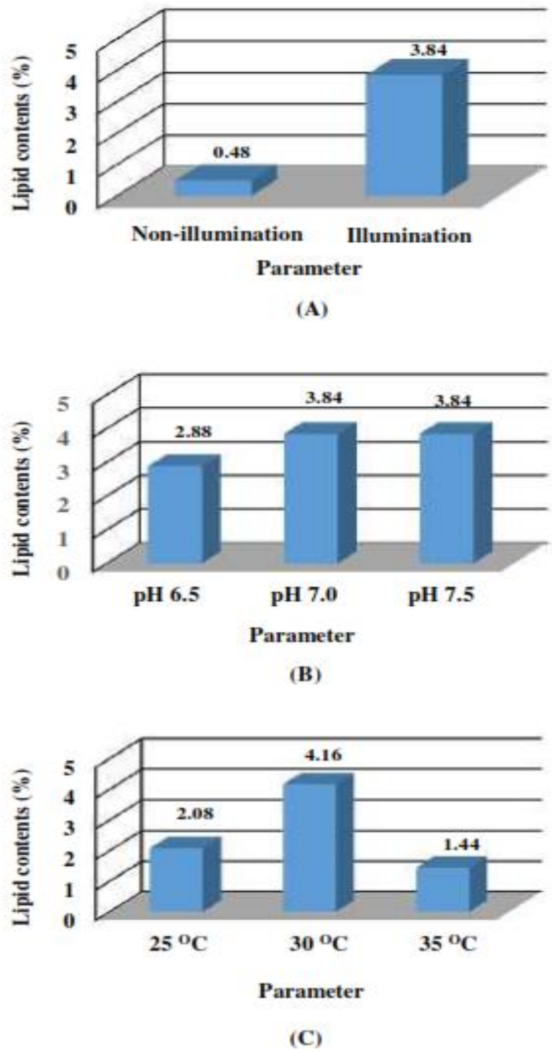

Figure 1. The effect of physical parameters on lipid content (%) in *Nostoc* sp. (A) light condition (B) pH condition (C) temperature condition

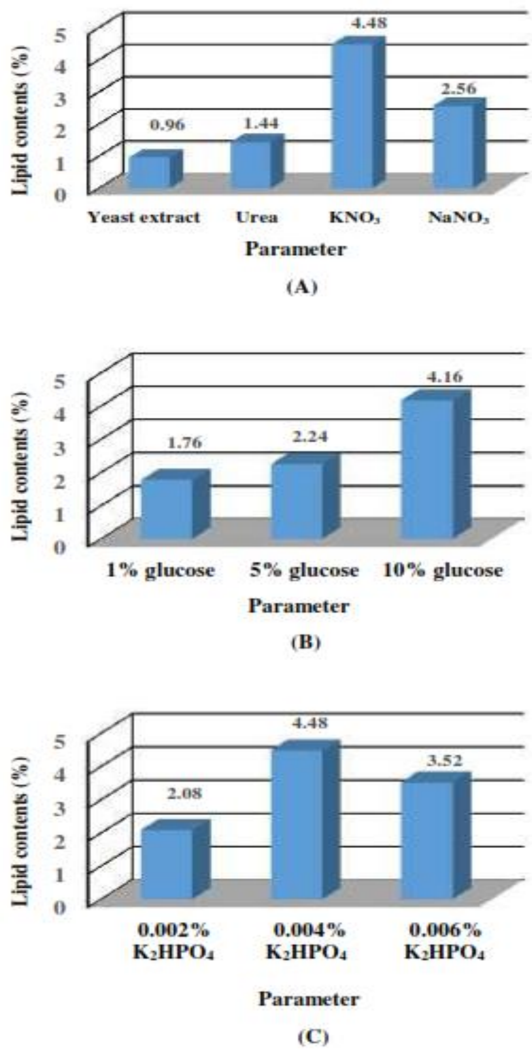

Figure 2. The effect of chemical parameters on lipid content (%) in *Nostoc* sp. (A) nitrogen sources (B) glucose concentration (C) dipotassium hydrogen phosphate concentration

4. Discussion

It had been reported that 3,000 species of microalgae were brought to identify their species and lipid production (5). Around 2,000 species of blue green algae had been identified and classified as 150 genera and 5 orders (6). Cyanobacteria is able to harvest light for photosynthesis and obtains important biomass (carbohydrate, protein and lipid) for living. Cyanobacteria had been mentioned by previous report for their low lipid accumulation (5). However, cyanobacteria is well known to has a high growth rate which is an advantage over other microalgae. Increasing of total lipid content in cyanobacteria are usually obtained under optimum conditions (7, 8). Algal species and environmental conditions are the factors which influence to growth and lipid content in cyanobacteria (9). High light intensity is not needed since many species of cyanobacteria may be harmed from this condition (10). It had been reported that low light intensity in turbid lake slightly affected to cyanobacteria growth. They still maintained their growth rate higher than other phytoplankton (11). Therefore, the high turbidity in large scale cultivation should be still regarded as potential promising source for lipid production from many cyanobacterial species. The optimal pH for *Nostoc* sp. growth is 7.0 which is the same as *N. commune* 1203 and *N. calcicola* 1205 demonstrated the optimum growth at pH 7.0 (12). Rai and Rajashekhar (13) also reported that *Nostoc commune* revealed maximum growth rate at pH 7.5 and its growth rate initiated to decline at pH value higher or lower than optimum pH. These results suggested that cyanobacteria preferred neutral to alkaline pH (12, 13). The temperature is physical parameter which is one of the important key to affect microalgae growth rate. Singh *et al*. (14) suggested that microalgae is able to grow in broad range of temperature but optimum temperature usually limit in narrow range approximately 20 to 30 degree celcious. Our *Nostoc* sp. gave the highest growth at 30 degree celcious. All optimum physical parameter could also induce *Nostoc* sp. to accumulate lipid reached to 4.16%. Then, the chemical parameters were investigated under optimum physical conditions. Potassium nitrate (KNO_3) and dipotassium hydrogen phosphate (K_2HPO_4) were the optimal nitrogen and phosphorus source, respectively which both conditions gave only 4.48% of the lipid content. From the results, the lipid value was rather low and it seems to not suit to be the lipid producer. However, *Nostoc* sp. could be further improved their growth rate and lipid production by studying the environmental stress such as nitrogen and phosphorus deprivation which shown in many microalgae that increase the lipid accumulation in their cells after entered to condition of nitrogen (15, 16) and phosphorus starvation (17). Also, it had been reported that some microalgae responded in better way when cultured under stress conditions such as high light intensity (18), high temperature (19) and salt stress (20). Moreover, fatty acid profiles of lipid should be observed to clarify the ratio of saturated and unsaturated fatty acids. Since, saturated and mono-unsaturated fatty acids were potential source for biodiesel production (18).

5. Acknowledgement

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