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THE EFFECT OF DIFFERENT PHOSPHATE CONCENTRATION ON GROWTH, LIPID PRODUCTIVITY AND METHYL PALMITATE METHYL ESTER PRODUCTION BY *NANNOCHLOROPSIS OCULATA*

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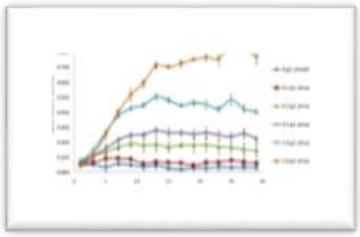
Graphical abstract



Abstract

This study reports the effect of different phosphate concentration ranging from 0 g/L, 0.1 g/L, 0.2 g/L, 0.5 g/L, 1.0 g/L and 2.0 g/L on the growth and lipid productivity of the microalgae *Nannochloropsis oculata*. Based on the result, it shows that, the microalgae growth increased as the concentration of phosphate added into the medium increased while the percentage of lipid produced was inversely proportional to increasing concentrations of phosphate. The highest amount of lipid produced was when the microalgae were cultured under 0.1 g/L phosphate which was 5.7%. Based on the standard analyzed using gas chromatography, the percentage of methyl palmitate methyl ester produced increased along with increasing concentration of phosphate until 0.5 g/L which was 11%, however further increase in phosphate concentration caused the lipid productivity to drop.

Keywords: Biodiesel, Microalgae, *Nannochloropsis oculata*, phosphate

Abstrak

Kajian ini melaporkan tentang kesan kepekatan fosfat yang berbeza dalam lingkungan 0 g/L , 0.1 g/L, 0.2 g/L, 0.5 g/L, 1.0 g/L sehingga 2.0 g/L terhadap pertumbuhan dan penghasilan lipid oleh mikroalga *Nannochloropsis* oculata. Berdasarkan keputusan eksperimen, pertumbuhan mikroalga meningkat dengan pertambahan kepekatan fosfat di dalam medium tetapi sebaliknya untuk penghasilan lipid. Jumlah lipid tertinggi dihasilkan apabila mikroalga dikultur di bawah 0.1 g/L fosfat iaitu sebanyak 5.7 %. Berdasarkan standard yang dianalisis dengan menggunakan gas kromatografi peratusan metil ester metil palmitat dihasilkan meningkat seiring dengan peningkatan kepekatan fosfat sehingga 1g/L iaitu 11 %, tetapi peningkatan lagi kepekatan fosfat menyebabkan peratusan metil ester metal palmitat menurun.

Kata kunci: Biodiesel, Microalgae, *Nannochloropsis oculata*, fosfat

1.0 INTRODUCTION

Fossil fuel is the main contributor for the emission and the accumulation of carbon dioxide in the environment [1].The first generation of biodiesel was produced from terrestrial crops but these types of source are not sustainable because it can cause the competition of land use and the destruction of world's forest. Waste oils are found at a very low cost with high free fatty acid [2] but frying processes can

change the structure of the cooking oil and effect the transesterification process [3]. This alternative energy source cannot satisfy the global demand on fuel for transportation. Productions of biodiesel from microalgae are getting more attention because it is more sustainable and convenient compared to conventional biodiesel derived from terrestrial crops [4]. Furthermore, lipid productivity of certain microalgae is very high with the lipid content of some species of microalgae exceeding more than 80% of its dry weight [5]. Several studies have been carried out to optimize the production of lipid in microalgae for biodiesel production. Most of these work are focused on optimization of growth and lipid productivity of microalgae such as studies to determine the effect of light intensity [6], carbon dioxide and nitrogen concentration [7, 8] and temperature [7] on the growth and lipid production of Nannochloropsis oculata. Other than light intensity, temperature, carbon dioxide and nitrogen concentration, phosphate deficiency was also found to be a suitable condition for lipid accumulation. Recent study had shown that Chlorella vulgaris increased lipid production during the phosphate starvation under autotrophic regimen, [9]. Some study also showed that phosphate is necessary for microalgae growth and phosphate deprivation has no effect on the lipid accumulation [10]. Therefore, in this study Nannochloropsi soculata growth was subjected under different phosphate concentration in order to evaluate the effect of phosphate concentration on overall growth and lipid accumulation of the culture.

2.0 EXPERIMENTAL

The *Nannochloropsis oculata* were cultured under different phosphate concentration ranging from 0 g/L, 0.1 g/L, 0.2 g/L, 0.5 g/L, 1.0 g/L and 2.0 g/L and done in three replicates in 1.0 L Erlenmeyer flask under constant illumination by florescence light at 2540 Lux with 12 hour light and 12 hours dark. The optical density were measured every two days [11] by using Jenway 6300 UV-spectrophotometer for 29 days. For the dry cell weight determination, 10 ml of culture samples were filtered through a pre-weighed glass microfiber filter paper [GFC, Whatmann] [12] that were heated in 80 °C oven to remove moisture.The filtered cells and filter paper were dried in an oven at 80 °C for at least 24 hours until constant weight were obtained [13].The microalgae were harvested after 29 days of cultured. The biomass of cultures were harvested by centrifugation then the wet cell mass were frozen overnight at -70°C under a vacuum [14]. The dried mass were grounded by mortar and pestle and subjected to sonication for 45 minutes after dissolved with 100 ml of distilled water. The total lipid were extracted by mixing chloroform-methanol 1:2 [v/v] with the sample in a proportion of 1 mL of sample to 3.75 mL mixed 1:2 [v/v] chloroform-methanol [15]. The mixtures then transferred into a separatory funnel and shaken for 5 minutes [14]. The chloroform layer containing the microalgae oil at the bottom layer of the separatory funnel was evaporated using eppendorf vacuum concentrator at 45 °C for 15 minutes. The extracted lipid then subjected to base catalyst transesterification [16]. The biodiesel produced were analyzed using gas chromatography. Methyl palmitate methyl ester [Sigma-Aldrich] was used as a standard in order to quantify the amount of fatty acid methyl ester produced.

3.0 RESULTS AND DISCUSSION

In order to evaluate the effect of different phosphate concentrations on *Nannochloropsis oculata* growth, the microalgae were cultured under six different phosphate concentration ranging from 0 g/L, 0.1 g/L, 0.2 g/L, 0.5 g/L, 1.0 g/L and 2.0 g/L. The growth curve was established by measuring the optical density of the microalgae culture every two days and the dry cell weight were done every four days for 29 days. The microalgae density increased as the concentration of phosphate in the medium increased. Based on Figure 1, the highest microalgae density obtained was at 2.0 g/L phosphate concentration and followed by microalgae cultured in medium containing 1.0 g/L, 0.5 g/L, 0.2 g/L, 0.1 g/L phosphate respectively. The lowest microalgae density obtained was when the microalgae cultured in the absence of phosphate. The dry cell weight measurement showed an increment pattern with increasing number of days as shown in Figure 2. The highest growth curve pattern based on the dry cell weight measurement was when *Nannochloropsis oculata* was cultured in medium containing 2.0 g/L phosphate while the lowest was when the microalgae cultured in medium without phosphate. The remaining microalgae that were not analyzed were harvested on the 29th day of culture. The harvested sample were then subjected to freeze-dried and the total biomasses were obtained by weighed the total harvested dried biomasses and the results were shown in Figure 3. Figure 3 shows that *Nannochloropsis oculata* growth is directly related to increasing concentration of phosphate with the highest growth registered at 2 g/L phosphate while the lowest is in the absence of phosphate. The generation time and specific growth rate were calculated based on the dry cell weight measurement that were performed every 4 days. Table 1 showed the specific growth rate and generation time for *Nannochloropsis oculata*. The highest specific growth rate was achieved when microalgae was cultured in medium containing 2.0 g/L phosphate followed by 1.0 g/L, 0.5 g/L, 0.2 g/L, 0.1 g/L and 0 g/ phosphate.

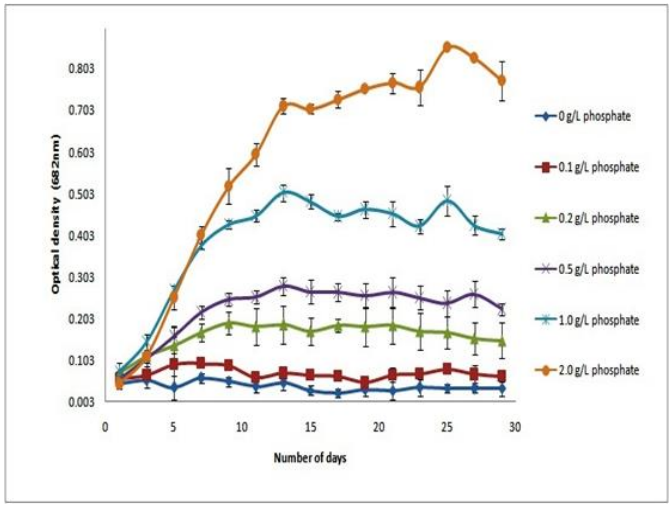


Figure 1 The optical density measurement of *Nannochloropsis oculata*

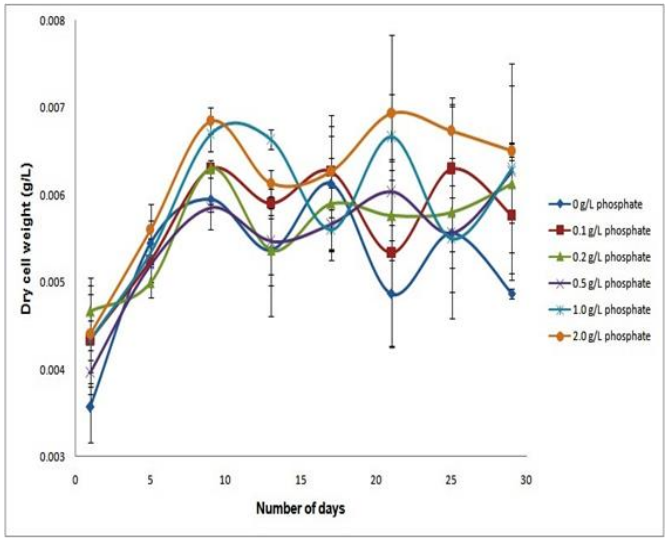


Figure 2 The dry cell weight measurement

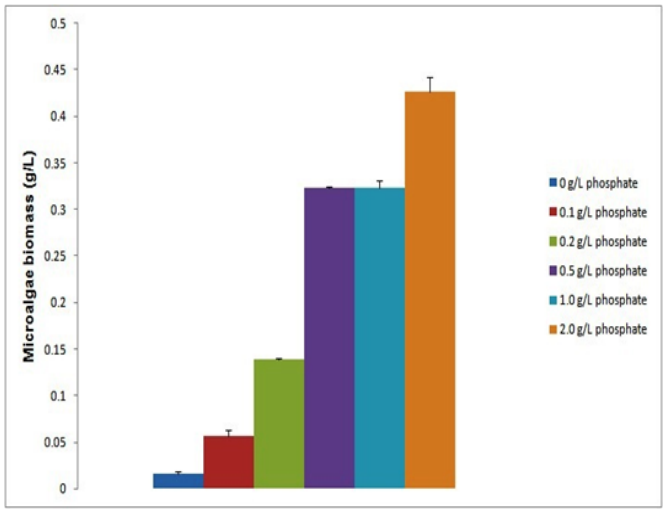


Figure 3 The total microalgae biomasses

The generation time were established from the specific growth rate obtained. The longest generation time was when *Nannochloropsis oculata was* cultured in medium containing 0 g/L which was 20 div.day^{-1}and the shortest generation time was when microalgae was cultured in medium containing 1.0 g/L and 2.0 g/L in which both concentrations took about 13 div.day^{-1}. The microalgae culture in medium containing 0.1 g/L and 0.2 g/L phosphate gave the same value which was 15 div.day^{-1} followed by microalgae cultured in medium containing 0.5 g/L which was 14 div.day^{-1}. Phosphate was shown to have positive effect on microalgae growth and this is in correlation to the results reported by Steffii and co-workers [2003], that showed phosphate had positive growth effect on microalgae species found in Perry pond [17] and this is because in all energy dependent process including respiration and growth require the one central energy carrier that plays a vital role is an energy rich phosphorus that is ATP [18]. For each ATP molecule contains three phosphate atoms that might had been contributed by the phosphate accumulation in the microalgae from the extracellular inorganic phosphate provided in the medium.

Table 1 The microalgae growth rate and population doubling time

Phosphate concentration [g/L]	Total microalgae biomasses [g/L]	Specific Growth rate	Generation time [div.day^{-1}]
0	0.01540	0.03388	20
0.1	0.05610	0.04678	15
0.2	0.13930	0.04754	15
0.5	0.22755	0.04856	14
1.0	0.32310	0.05447	13
2.0	0.42640	0.05533	13

Based on Table 2, the percentage of lipid produced decreased as the amount of phosphate increased. The highest amount of lipid produced was when microalgae were cultured under 0.1 g/L phosphate which was 5.7% lipid and followed by 0 g/L phosphate which was 4.55%. The highest lipid productivity was when microalgae were cultured under low phosphate concentration. This is because, microalgae increased the lipid storage under phosphate deficiency [19]. The formula for percentage of lipid produce is

$$\frac{\text{Total lipid}}{\text{Total biomass}} \times 100$$

Table 2 The percentage of lipid and methyl palmitate methyl ester produced by the microalgae

Phosphate concentration [g/L]	Percentage of lipid produced [%]	Percentage of methyl palmitate methyl ester produced [%]
0	4.5455	0.2727
0.1	5.7041	0.3633
0.2	4.3790	0.5697
0.5	3.6475	1.1051
1.0	2.1046	0.9447
2.0	1.0553	0.3822

The $NADP^+$ acts as an electron acceptor for photosynthesis that cannot be shut down entirely. Under nutrient starvation, the depletion of $NADP^+$ can be fatal to cell as this can damage the cell components. The fatty acid biosynthesis increased the production of fatty acids [stored in Triacylglycerol] which is a process that consumed NADPH and restore back the $NADP^+$ at once [20]. Methyl palimitate methyl ester was used as standard for gas chromatography analysis because high saturated fatty acid such as methyl palmitate methyl ester was found to be suitable candidate for biodiesel [21] and the chromatogram for metyl palmitate metyl ester standard was shown in Figure 4. Gas chromatography analysis showed that the percentage of methyl palmitate methyl ester produced increased as the concentration of phosphate increased until 0.5 g/L but, further increase of phosphate concentration had caused the decrease of methyl palmitate methyl ester production. The chromatogram for the highest of methyl palmitate methyl ester detection in biodiesel sample from microalgae that were culture in medium containing 0.5 g/L phosphate was shown in Figure 5. Methyl palmitate methyl ester is an important component for the production of high quality biodiesel because high content of saturated fatty acid give oxidative stability to biodiesel and improve combustion [21].

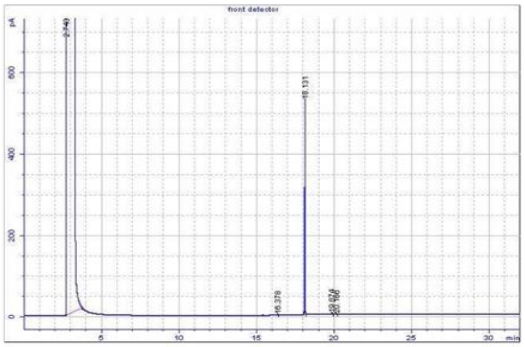


Figure 4 Methyl palmitate metyl ester standard chromatogram for gas chromatography analysis

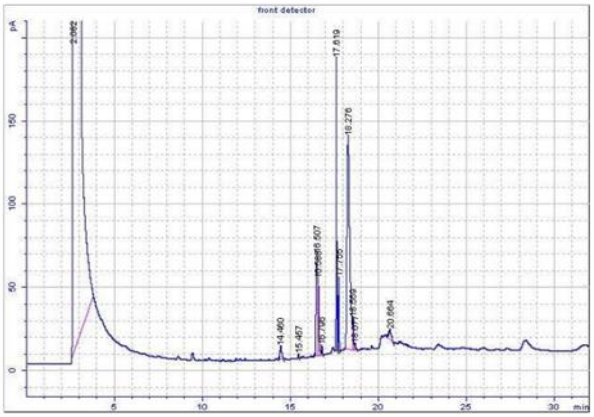


Figure 5 The chromatogram for the highest methyl palmitate methyl ester detection using gas chromatogram

4.0 CONCLUSION

Phosphate had shown to have positive effect on *Nannochloropsis oculata* growth while the percentage of lipid produced was inversely proportional with increasing phosphate concentrations. The highest methyl palmitate methyl ester produced was when microalgae cultured under 0.5 g/L phosphate but, further increase of phosphate concentration had cause the decreased of methyl palmitate methyl ester production.

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