Enhancement of lipid production in two marine microalgae under different levels of nitrogen and phosphorus deficiency

Nurul Salma Adenan¹, Fatimah Md. Yusoff¹,2,*, Srikanth Reddy Medipally¹ and M. Shariff¹, 3

¹Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
²Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
³Aquatic Animal Health Unit, Faculty of Veterinary Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

*Corresponding Author E-mail: fatimahyus@gmail.com

Abstract

Microalgae are important food sources for aquaculture animals. Among the different factors which influence the biochemical composition of microalgae, nitrogen and phosphorus are two of the most important nutrient sources for growth and development. The present study aimed to assess the effects of nitrogen and phosphorus deficiency on lipid production of Chlorella sp. and Chaetoceros calcitrans. Early stationary phase culture of these species were exposed to different stress levels of nitrogen and phosphorus (25%, 50% and 75% of the full NO₃-N and PO₄-P concentration in the Conway media), and solvent extraction and gas-liquid chromatography methods were performed for analysis of lipid and fatty acid composition. The results revealed that lipid production in these two species significantly increased (P<0.05) as nitrogen and phosphorus decreased. The fatty acid proportion remained unaffected under nitrogen deficiency, while phosphorus limitation resulted in a decrease of saturated fatty acids and promoted a higher content of omega-3 fatty acids in these species. The protein and carbohydrate levels were also altered under limited nutrients. Therefore, these conditions could be used for enhanced lipid production in microalgae for aquaculture and other industrial applications.

Key words

Chaetoceros calcitrans, Chlorella, Lipid, Marine microalgae, Nutrient stress, Omega-3 fatty acids

Introduction

Microalgae have diverse applications in biofuels, aquaculture, nutraceuticals, pharmaceuticals, cosmetics and food industries. They are found in all the aquatic environments and play an important role in minimizing global warming by fixing a substantial amount of atmospheric carbon dioxide (Zhu et al., 2013). Microalgae play a prominent role in the aquaculture industry by stabilizing water quality and eliminating metabolic byproducts (Khatoon et al., 2007). Moreover, live microalgae are conventionally used directly as feed for aquaculture animals or as bioencapsulated feed in live organisms such as copepods and cladocerans (Khatoon et al., 2013). About 30% of world algae production is used as aquaculture animal feed (Becker, 2004). Nutritional composition such as proteins, carbohydrates and lipids of microalgae is three times more than those derived from fish and plants (Spolaore et al., 2006).

High lipid content in microalgae has received attention due to its varied applications (Rawat et al., 2013). High polyunsaturated fatty acids (PUFA) content, in particular eicosapentaenoic acid (EPA), docosapentaenoic acid (DHA), can provide a high quality nutritional package for different stages of aquaculture animals (Khatoon et al., 2013; Banerjee et al., 2011). However, there is a need to understand factors that can influence the lipid and fatty acid composition. Growth requirements such as nutrients, pH, light intensity, salinity and temperature all have an impact on
maximizing the beneficial output from microalgae (Banerjee et al., 2011). However, one of the major strategies for enhancing lipid production in microalgae is through nutritional stress, such as limiting nitrogen and phosphorus supplementation during cell growth (Sugimoto et al., 2008).

Nitrogen and phosphorus limitation has been shown to cause high levels of lipid accumulation in a number of microalgae species (Illman et al., 2000; Khozin-Glidberg and Cohen, 2006; Li et al., 2008a). However, this approach is associated with reduced cell divisions (Ratledge, 2002). Along with lipids, microalgae species are also able to accumulate various amounts of cell constituents such as carbohydrates, proteins and fatty acids (Uslu et al., 2011). To date, very limited research has been carried out on methods to potentially enhance the lipid production of tropical marine microalgae. Therefore, the present study was undertaken to evaluate the effect of nitrogen and phosphorus deficiency on lipid production in Chlorella sp. and Chaetoceros calcitrans (Malaysian tropical marine microalgae).

Materials and Methods

Algae cultures: Chlorella sp. (UPMC-A0013) and Chaetoceros calcitrans (UPMC-A0010) were obtained from the Microalgae Culture Collection of Marine Biotechnology Laboratory, Institute of Bioscience, Universiti Putra Malaysia.

Experimental design: Early stationary phase cultures of Chlorella sp. and C. calcitrans were used as inocula. Ten percent inocula were inoculated into 500 ml Erlenmeyer flasks containing 300 ml of Conway medium with different concentrations of nitrogen [0% - N (initial concentration), 25% - (N), 50% (N), and 75% (N)] and phosphorus [0% (P , initial concentration), 25% (P), 50% (P) and 75% (P)]. Salinity and growth temperature used were 30% and 25 °C, respectively. Cell density was inferred in the form of cell number, optical density and biomass were measured daily (Lavens and Sorgeloos, 1996). The cultures were sparged with filter-sterilized air by using air pump without additional carbon dioxide and were continuously illuminated by three fluorescence lamps of 36W each (600-800 lux). The treatments were repeated thrice.

Growth monitoring: Cell concentrations of microalgae cultures were determined by using optical density, cell counts and biomass estimation. The absorbance was read at a wavelength of 540 nm by Shimadzu spectrophotometer model UV-1601. Cell counts were accomplished by a Neubauer haemocytometer.

Specific growth rates were calculated as described in the following formula:

\[
\mu (d^{-1}) = \frac{\ln (F_t / F_{t_0})}{t - t_0}
\]

Where, \( F_t \) = Biomass at the time of harvest \( (t) \) and \( F_{t_0} \) = Biomass at times zero, \( (t_0) \) (Guillard, 1973)

Lipid and other biochemical analyses: One ml of culture was taken into 1.5 ml Eppendorf tube 4 days after the stress was applied and centrifuged at 5000 rpm for 3 min. The harvested biomass of Chlorella sp. and C. calcitrans were washed with 0.9 % NaCl to eliminate non-biological material such as mineral salt precipitate. The biomass was then stored at -20°C for the analyses of total lipid and other biochemical composition. Total lipids were determined using modified protocol of Marsh and Weinstein (1966) with 1:2 ratio of chloroform : methanol as the solvent. The quantity of extracted lipids was determined by spectrophotometer at 375nm. For estimation of fatty acid, FAMEs analysis was carried out by using gas-liquid chromatography (Hewlett-Packard, Model 5890, Avondale, PA). It was equipped with FID detector, SP-2330 fused silica capillary column (30mm, 0.25mm ID, 0.20 μm film tickness, Supelco, Inc., Bellefonte, PA); high purity nitrogen and hydrogen (> 99.9%, Malaysian Oxygen Berhad, Malaysia) was used as a carrier gas. The column temperature was programmed from 150°C to 190°C at ramp rate of 3°C min⁻¹. Injector and detector temperatures were adjusted to 250°C and 280°C, respectively. Peak area was determined using a HP-3393A integrator (Hewlett Packard, Avondale, PA). Total protein was estimated by following the method of Lowry et al., (1951). Carbohydrate extraction was performed by using phenol-sulfuric acid method (Dubois et al., 1956) and the quantity was determined by spectrophotometer.

Statistical analysis: Data analysis was carried out using one-way ANOVA Statistical Package for Social Sciences (SPSS Version 15.0).

Results and Discussion

Nitrogen deficiency: Marine Chlorella sp. and C. calcitrans exhibited high cell density when they were cultured under normal growth condition where sufficient nutrient concentrations stimulated the optimal growth of microalgae. In the study, the growth rate of Chlorella sp. decreased
Enhanced lipid production in marine microalgae under nutrient stress

In the present investigation, decreased nitrogen concentration showed significant changes (P<0.05) in the lipid content of *Chlorella* sp. and *Chaetoceros calcitranis*. Li et al. (2008a) reported accumulation of storage lipids in green algae during nitrogen deficient conditions. There was a significant decrease (P<0.05) in the total lipid production of *Chlorella* sp. to 19.2% of d.w.t. upon reduction of nitrogen concentration to 25% (N\(i\)) (Fig. 1). There was no change (P>0.05) in percentage of lipid production when exposed from N\(i\) to N\(o\), however, when nitrogen was reduced to 75% (N\(l\)) a significant increase (P<0.05) in lipid content was observed by 11.0% of d.w.t. In *C. calcitrans*, production of total lipid in N\(i\) was 26.0% of d.w.t. and increased to 5.0% of d.w.t. at N\(l\) (Fig. 1). Lipid production increased significantly (P<0.05) by 7.0% and 10.0% of d.w.t. at N\(25\) and N\(75\), respectively. Highest lipid production was observed when microalgae were exposed to a 75.0% reduction of the nitrogen concentration at the early stationary phase (Fig. 1).

Effect of nitrogen deficiency on total lipid production:
Nitrogen deficiency generally enhances lipid production in many microalgae (Illman et al., 2000). Normally sufficient nutrient availability produces more proteins and enhances cell proliferation. When nutrients are depleted, cell growth is suppressed and enters into the stationary phase; during this phase accumulated carbons gets transformed into lipids (Cheng and Orden, 2011).

Table 1: Specific growth rates of *Chlorella* sp. and *Chaetoceros calcitranis* in response to different levels of nitrogen reduction

<table>
<thead>
<tr>
<th>KNO(_3) concentration (mg l(^{-1}))</th>
<th>Growth rate (μ day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(i) (13.86)</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>N(o) (10.38)</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>N(l) (6.93)</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>N(o) (3.46)</td>
<td>0.30 ± 0.04</td>
</tr>
</tbody>
</table>

Data are reported as mean ± standard error of triplicates. Means in column with different letters (a-d) are significantly different at P< 0.05, one-way ANOVA

Table 2: Fatty acid profiles of *Chlorella* sp. and *Chaetoceros calcitranis* in response to 75.0% nitrogen reduction

<table>
<thead>
<tr>
<th>Fatty acid (% of total fatty acid)</th>
<th><em>Chlorella</em> sp.</th>
<th><em>Chaetoceros calcitranis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.0 ± 0.5</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>26.8 ± 1.0</td>
<td>24.5 ± 0.3</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.1 ± 0.3</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>C18:0</td>
<td>5.8 ± 0.8</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>C18:1</td>
<td>11.0 ± 0.7</td>
<td>8.4 ± 0.4</td>
</tr>
<tr>
<td>C18:2 n-6</td>
<td>23.2 ± 0.3</td>
<td>23.9 ± 1.3</td>
</tr>
<tr>
<td>C18:3 n-3</td>
<td>13.0 ± 0.1</td>
<td>14.8 ± 0.7</td>
</tr>
<tr>
<td>C20:4 n-6</td>
<td>---</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>C20:5 n-3</td>
<td>---</td>
<td>22.6 ± 0.7</td>
</tr>
<tr>
<td>Total Saturated</td>
<td>33.5 ± 0.8</td>
<td>31.8 ± 1.2</td>
</tr>
<tr>
<td>Total Unsaturated</td>
<td>50.3 ± 1.2</td>
<td>51.1 ± 0.6</td>
</tr>
<tr>
<td>Total PUFA n-3</td>
<td>13.7 ± 0.6</td>
<td>14.8 ± 0.4</td>
</tr>
<tr>
<td>Total PUFA n-6</td>
<td>23.2 ± 0.2</td>
<td>23.9 ± 0.1</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>36.8 ± 1.6</td>
<td>38.7 ± 0.5</td>
</tr>
</tbody>
</table>

Data are reported as mean ± standard error of triplicates. Means in row with different letters (a-b) are significantly different P<0.05, one-way ANOVA.
There was relatively a small increase in arachidonic acid (C20:4 n-6) in 75.0% nitrogen deprived medium, whilst, linoleic acid (C18:2n-6) significantly decreased. Harrison et al. (1990) reported a significant change in the fatty acid proportion of marine microalgae when subjected to nitrogen stress. However, this depended on other factors such as isolates and culture media; the origin of isolates could also influence the fatty acid production (Mata et al., 2010). In the present study, total ω-3 and ω-6 PUFA production in Chlorella sp. was 10.0% higher than that of C. calcitrans, which suggested that these microalgae species had similar quality as a food source in aquaculture industries.

Effect of nitrogen deficiency on carbohydrate and protein contents:

There was relatively a small increase in arachidonic acid (C20:4 n-6) in 75.0% nitrogen deprived medium, whilst, linoleic acid (C18:2n-6) significantly decreased. Harrison et al. (1990) reported a significant change in the fatty acid proportion of marine microalgae when subjected to nitrogen stress. However, this depended on other factors such as isolates and culture media; the origin of isolates could also influence the fatty acid production (Mata et al., 2010). In the present study, total ω-3 and ω-6 PUFA production in Chlorella sp. was 10.0% higher than that of C. calcitrans, which suggested that these microalgae species had similar quality as a food source in aquaculture industries.

Effect of nitrogen deficiency on carbohydrate and protein contents:
The study showed that the carbohydrate composition was highest proximate composition in Chlorella sp. with an average value of 42.0% of d.w.t. at initial (N) nitrogen concentration (Fig. 2). However, a reduction in nitrogen resulted in a significant decrease (P<0.05) in the carbohydrate composition (6.0% of d.w.t.) Meanwhile,

Table 4: Fatty acid profiles of Chlorella sp. and Chaetoceros calcitrans in response to 75.0% phosphorus reduction

<table>
<thead>
<tr>
<th>Fatty acid (% total fatty acid)</th>
<th>Chlorella sp. P (5.2 mg l⁻¹)</th>
<th>Chlorella sp. Pₕ (1.33 mg l⁻¹)</th>
<th>Chaetoceros calcitrans P (5.2 mg l⁻¹)</th>
<th>Chaetoceros calcitrans Pₕ (1.33 mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.0 ± 0.2²</td>
<td>nd*</td>
<td>31.2 ± 0.3</td>
<td>18.9 ± 0.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>26.8 ± 0.1¹</td>
<td>15.2 ± 1.2²</td>
<td>15.5 ± 0.1</td>
<td>14.4 ± 0.3</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.1 ± 0.1²</td>
<td>4.2 ± 0.1¹</td>
<td>26.5 ± 0.3</td>
<td>19.4 ± 0.1</td>
</tr>
<tr>
<td>C18:0</td>
<td>5.8 ± 0.2²</td>
<td>9.3 ± 0.0¹</td>
<td>4.1 ± 0.2</td>
<td>7.5 ± 0.1</td>
</tr>
<tr>
<td>C18:1</td>
<td>11.0 ± 1.1¹</td>
<td>3.1 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>C18:2 n-6</td>
<td>23.2 ± 0.4²</td>
<td>17.9 ± 1.4</td>
<td>3.9 ± 1.2</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>C18:3 n-3</td>
<td>13.0 ± 0.2²</td>
<td>19.5 ± 0.3</td>
<td>0.9 ± 0.5</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>C20:4 n-6</td>
<td>—</td>
<td>—</td>
<td>0.8 ± 0.2</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>C20:5 n-3</td>
<td>—</td>
<td>—</td>
<td>22.6 ± 1.2</td>
<td>29.3 ± 0.5</td>
</tr>
<tr>
<td>Total Saturated</td>
<td>33.5 ± 1.2²</td>
<td>24.5 ± 0.8</td>
<td>50.8 ± 1.2</td>
<td>40.8 ± 0.5</td>
</tr>
<tr>
<td>Total Unsaturated</td>
<td>50.3 ± 0.4</td>
<td>44.7 ± 1.0</td>
<td>55.8 ± 0.8</td>
<td>62.3 ± 0.0</td>
</tr>
<tr>
<td>Total PUFA n-3</td>
<td>13.7 ± 1.0</td>
<td>19.5 ± 0.2</td>
<td>23.5 ± 1.5</td>
<td>30.5 ± 0.1</td>
</tr>
<tr>
<td>Total PUFA n-6</td>
<td>23.2 ± 0.1</td>
<td>17.9 ± 0.8</td>
<td>4.7 ± 0.4</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>36.8 ± 0.0</td>
<td>37.4 ± 0.4</td>
<td>28.2 ± 1.0</td>
<td>38.9 ± 0.2</td>
</tr>
</tbody>
</table>

*nd = Not detected.

Data are reported as mean ± standard error of triplicates. Means in row with different letters (a-b) are significantly different at P<0.05, one-way ANOVA.
protein content of *Chlorella* sp. decreased significantly (P<0.05) from 33.0% (N) to 28.0% (N<sub>n</sub>) d.w.t. In *C. calcitrans*, there was in significant change (P>0.05) in carbohydrate content in response to nitrogen stress (Fig. 2), although the protein content decreased significantly (P<0.05) from 38.0% (N) to 27.0% d.w.t. (at N<sub>n</sub>).

Biochemical properties affect the quality of marine microalgae, which subsequently influence the productivity of aquaculture organisms. In the study, lipid production was increased after subjecting the microalgae to a reduction to nitrogen by 75.0% (N<sub>n</sub>) (Fig. 1). However, this reduction resulted in a decrease in protein and carbohydrate contents in *Chlorella* sp. This condition was probably due to the availability of insufficient nitrogen required for protein and carbohydrate metabolic pathways (Fig. 2). Similar results were obtained for *Chlorella vulgaris* when cultured in a nitrogen deprived medium (Mutlu *et al*., 2011). In case of *C. calcitrans*, nitrogen deprivation caused a drastic drop in the protein content (Fig. 2). This was probably associated with the amount of nitrogen availability (Otero and Fabregas, 1997). This suggests that tropical marine microalgae were able to produce high amount of lipid efficiently under nitrogen deprived conditions without affecting the fatty acid profiles and nutrient composition in *Chlorella* sp. and *C. calcitrans*. Although there was a decrease in cell proliferation, the biomass obtained was still sufficient to be used in the aquaculture industry.

**Phosphorus deficiency**: Phosphorus deficiency acts as a limiting factor for the growth of plants and microorganisms and, to sustain these limiting conditions, they adopt different strategies to cope with changing environments (Hölzl and Dörmann, 2007). In this study, the dry weight of both the microalgae in phosphorus deprived cultures were found to be lower as compared to non-deprived cultures. Similar results were obtained for *Chlorella vulgaris* when cultured in a nitrogen deprived medium (Mutlu *et al*., 2011). In case of *C. calcitrans*, reduction to nitrogen by 75.0% (N<sub>n</sub>) (Fig. 1).

**Fig. 1**: Total lipid production of *Chlorella* sp. and *Chaetoceros calcitrans* in response to different levels of nitrogen reduction. Each error bar represents mean ± standard error of triplicates (n=3). Mean values with different letters (a-c) are significantly different at P<0.05, one-way ANOVA.

**Fig. 2**: Proximate composition in *Chlorella* sp. and *Chaetoceros calcitrans* in response to 75.0% nitrogen reduction. Each error bar represents mean ± standard error of triplicates (n=3). Mean values with different letters (a-e and a-b) are significantly different at P<0.05, one-way ANOVA.
were found in *Monodus subterraneus* (Khozin-Goldberg and Cohen, 2006). Usually, there is a high demand for phosphorus in fast growing microalgae than slow growing species (Ferraro-Filho et al., 2003). Here, *Chlorella* sp. and *C. calcitrans* are fast growing species and therefore, are affected more under phosphorus limiting conditions. Furthermore, phytoplankton has the capacity to rearrange cellular phosphorus for sustaining maximal growth (Ji and Sherrell, 2008). In the present study, this condition was demonstrated practically in the diatom, which showed a slight decrease in the growth rates as compared to *Chlorella* sp.

**Effect of phosphorus deficiency on total lipid content:**
Phosphorus availability is known to have significant effects on lipid biosynthesis in microalgae and is involved in the formation of membrane phospholipids for animals and yeast (Khozin-Goldberg and Cohen, 2006; Reitan et al., 1994). However, when there is limited access of lipid in plant and algae, cell membranes are dominated by galactolipids as an approach to minimize the dependence on phosphate (Khozin-Goldberg and Cohen, 2006). Indeed, phosphate limitation results in an increase of lipid in plants; *Arabidopsis thaliana* (Härtel et al., 2000), photosynthetic bacterium, *Rhodobacter sphaeroides* (Benning et al., 1995), freshwater alga; *Desmodesmus subspicatus* and soil alga; *Chlamydomonas reinhardtii* (Lind et al., 2004).

Under phosphorus deficient conditions, both species exhibited significantly lower (P<0.05) growth in terms of cell number and dry weight along with high lipid production (Table 3). Phosphorus limitation increased the lipid production in both these species, but the type and quantity of lipids produced varied under different stress levels (Fig. 3). In the initial phosphorus concentration (P), total lipids in *Chlorella* sp. was up to 23.0% of d.w.t. and after exposure to 25.0% reduction (P), lipid production declined to 18.0% of d.w.t. whilst a 50.0% reduction resulted in insignificant changes (P>0.05) in total lipid as compared to P. On the other hand, a 75.0% reduction resulted in a significant increase (P<0.05) of total lipids up to 32.0% of d.w. Under similar growth conditions, the total lipid production in *C. calcitrans*...
at $\text{P}_i$ was 26.0% of d.w. (Fig. 3). After exposure to $\text{P}_i$, no significant change ($P>0.05$) in the total lipid production was observed. However, an increase ($P<0.05$) of the total lipid production up to 29.0% and 35% of d.w. was observed after reducing phosphorus to $\text{P}_i$ and $\text{P}_f$, respectively. The response in lipid production varied between these two species and the phosphorus concentration also influenced its productivity. A minimum phosphorus concentration ($\text{P}_i$) appeared to be the best concentration that showed a significant increase ($P<0.05$) in lipid production in both the species as compared to normal culture. This appeared to show that a 25.0% to 50.0% reduction in phosphorus did not provide enough stress on the cultures as observed previously in *Monodus subterraneus*, which produced highest lipid under low phosphorus concentrations (Khizin-Goldberg and Cohen, 2006).

**Effect of phosphorus deficiency on fatty acid composition:** In *Chlorella* sp. phosphorus reduction by 75.0% significant by decreased the proportion of palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2n-6) (Table 4). However, similar culture conditions resulted in a significant increase (P<0.05) in lipid production in both the species as compared to normal culture. This appeared to show that a 25.0% to 50.0% reduction in phosphorus did not provide enough stress on the cultures as observed previously in *Monodus subterraneus*, which produced highest lipid under low phosphorus concentrations (Khizin-Goldberg and Cohen, 2006).

**Effect of phosphorus reduction on carbohydrate and protein contents:** Elevated protein and carbohydrate content have frequently been reported in studies of low phosphorus media (Sigee et al., 2007). However, in the present study a significant decrease ($P<0.05$) in the protein and carbohydrate content was observed in *C. calcitrans* after exposure to a reduction in phosphorus by 75%, as observed elsewhere (Mutlu et al., 2011). The carbohydrate composition of *Chlorella* sp. and *C. calcitrans* was high in the non-deprived medium ($P_i$) for both species (Fig. 4). However, the proportion of carbohydrates decreased significantly ($P<0.05$) in these species by 13.0% and 5.0% of d.w. at 75 % ($P_f$) reduction, respectively. Meanwhile, the protein content in *Chlorella* sp. increased to 40.0% d.w.t. when cells were subjected to $\text{P}_f$. On the other hand, similar stress conditions resulted in a significant decrease ($P<0.05$) in the protein proportion from 42.0% to 38.0% of d.w. for *C. calcitrans*. Phosphorus deprivation enhanced lipid production in *Chlorella* sp. and *C. calcitrans* even though it resulted in decreased biomass. This set of culture conditions is applicable in increasing the total lipid production and omega-3 fatty acid in both tropical marine microalgae species.

**Acknowledgments**

This research was funded by the Ministry of Science, Technology and Innovation Malaysia (MOSTI) through Johor State Biotechnology Satellite Project No., (BSP/J/BTK/001(4)) and e-ScienceFund Project No. 04-01-04-SF1012.

**References**


Ferrãro-Filho, A.S., C. Fileto, N.P. Lopes and M.S. Arca: Effects of essential fatty acids and N and P-limited algae on the growth of


