Biomass and lipid production of marine diatom *Amphiprora paludosa* W. Smith at different nutrient concentrations

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Abstract

A feasible and alternative source of biological material for the production of biodiesel is represented through microalgae. Production of biodiesel from microalgae has been attracting the attention of researcher world-wide. A number of scientific reports have described the advantages of biodiesel production from microalgae, compared with that from other feedstock. This paper deals with the growth, total lipid content and fatty acid methyl ester (FAMEs) of a marine diatom *Amphiprora paludosa* W. Smith at different concentrations of sodium silicate, sodium nitrate, Fe-EDTA and sodium phosphate under laboratory conditions. The alga showed maximum biomass production of 330 mg L^{-1} at 0.075mM of sodium phosphate whereas maximum total lipid content of 65.64% dry weight was recorded in the medium amended with 0.026mM of Fe-EDTA. The total lipid obtained from the alga was converted into biodiesel through transesterification and its fatty acid composition were analysed through GC-MS.

1. Introduction

Biodiesel is one of the most promising renewable fuels that have achieved remarkable success worldwide according to the World Bank report (2008). Oil-rich microalgae have been demonstrated to be a promising alternative source of lipids for biodiesel production (Walker *et al.*, 2005; Chisti, 2007; Li *et al.*, 2008; Song *et al.*, 2008; Wang *et al.*, 2008). There seems to be little doubt that fast-growing microalgae should be able to provide enough renewable biofuels as a replacement for fossil fuels (Li *et al.*, 2008). Global warming and exhaustion of fossil fuels are major environmental and economical issues the world is facing today. Due to the limited stocks of fossil fuels and increasing emission of greenhouse gases into the atmosphere from the combustion of fossil fuels, research has begun to focus on alternative biomass-derived fuels (Scurlock *et al.*, 1993; Kosaric and Velikonja 1995). Microalgae have the ability to grow rapidly, and to synthesize and accumulate more amounts of neutral lipid (20-50% of dry weight), mainly in the form of triacylglycerol, TAG and stored in cytosolic lipid bodies (Day *et al.*, 1999; Hu *et al.*, 2008). Some species of diatoms such as *Chaetoceros muelleri* have been considered to be an ideal source of neutral lipids suitable for biodiesel production (Mcginnis *et al.*, 1997; Illman *et al.*, 2000). Biodiesel has better properties than that of petroleum diesel because biodiesel is renewable, biodegradable, non-toxic and essentially free of sulphur and aromatic compounds. Recent investigation has indicated that the use of biodiesel can reduce 90% pollution (Sharp 1996).

Microalgal lipids are mostly neutral lipids due to their lower degree of unsaturation and their accumulation in the microalgal cell at the early or late end of the growth phase. This makes microalgal lipids a potential diesel fuel substitute (Casadevall *et al.*, 1985; Chisti, 2007). These lipids are essential nutrients for cell growth. Their main role is to make up a reserve of metabolic energy (Dempster and Sommerfeld, 1998), and to provide among other fatty acids, long-chain polyunsaturated fatty acids (PUFAs), which function as a key part of the biomembrane structures (Salhi, 1994). Microalgae are an optimal source of PUFAs, and new industrial scale culture systems are being developed in order to produce large amounts of biomass. The technical advances suggest that the industrial production of biodiesel from microalgal oils may be feasible in the near future. However, an economic utilization system needs to be standardizing for the optimal production of the resources. In this way, manipulation of culture conditions (physico-chemical factors) represents a solution to increase the lipid yield (Roessler, 1990). As

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lipid production by microalgae is not regulated by environmental factors in the same manner for all species (Chelf, 1990), a control is required. In this present study a marine diatom *Amphiprora paludosa* W. Smith isolated from Kovelong coast near Chennai, was chosen and experiment were conducted enhance lipid content at different concentrations of silicate, nitrogen, phosphorus and iron in the laboratory conditions.

2. Material and Methods

2.1. Marine diatom

The algal samples were collected along the Kovelong coast, near Chennai Tamil Nadu, India, using phytoplankton net of 25µm in 2009. The samples contained diatoms were transferred to f/2 (Guillard and Ryther 1962) medium and kept at 24±1ºC in a thermostatically controlled room, illuminated with cool white florescence lamps at an intensity of 30µEm $^{-2}$s $^{-1}$, and 12:12 h of light/ dark regime. After 5 days the samples were serially diluted up to 10^{-10} and 0.1mL spread on 2% f/2 agar medium. The cyanobacterial contaminants were eliminated by treating them with 3000 ppm of the antibiotic, Streptomycin sulphate for 30 min under 30µEm $^{-2}$s $^{-1}$ light intensity and transferred to antibiotic free basal medium (Rengasamy *et al.* 1987). Among the diatoms *Amphiprora paludosa* W. Smith was isolated and identified based on its morphological characters (Venkataraman, 1939).

2.2. Experiments

Amphiprora paludosa was grown at different concentrations of sodium silicate: 0.035 mM, 0.070 mM, 0.105 mM (control), 0.140 mM, 0.175 mM, 0.210 mM and 0.245 mM; sodium nitrate: 0.760 mM, 0.820 mM, 0.882 mM (control), 0.940 mM, 1.00 mM, 1.10 mM and 1.12 mM; Fe-EDTA: 0.009 mM, 0.0140 mM (control), 0.017 mM, 0.020 mM, 0.023 mM and 0.026 mM and sodium phosphate: 0.025mM, 0.0362 mM (control) 0.050 mM, 0.058 mM, 0.067 mM and 0.075 mM.

Thirty mL of optimally grown culture was inoculated in 270 mL^{-1} f/2 (Guillard and Ryther, 1962) medium and kept under laboratory conditions. Experiments were conducted for a period of 21 days. At every three days of interval dry biomass and total lipid (Folch *et al.* 1957) were estimated and recorded.

2.3. Dry biomass

The culture was pelleted by centrifugation at 5000 rpm for 10 minutes. The pellet was washed three times with ammonium formate 0.65M (Moheimani, 2005) in order to remove the salt content. Then it was transferred to pre dried GF/C glass fiber filter paper and keep at 60°C for overnight in a hot air oven and cooled in a desiccator before weighing dry biomass.

2.4. Extraction and purification of total lipids

Lipids were extracted by the modified procedure as described by Bligh and Dyer (1959). The cells were homogenized with chloroform: methanol: water (2:1:0.8, v/v/v) for 2 min. An equal volume of chloroform and distilled water was then added, to bring the final ratio of the mixture to 2:2:1.6 and the mixture were then homogenized for 1 min. The chloroform layer containing lipid fraction was separated, and the alcoholic layer, contained the residues, were re-extracted twice with methanol and chloroform (1:2, v/v).

The chloroform layers were pooled and subjected to a "Folch wash" to remove all non-lipid contaminants (Christie, 1992). The mixture was washed with one-fourth of the volume of 0.88% (wt/v) potassium chloride, followed by methanol/saline solution (1:1, v/v). The purified chloroform extract was then evaporated under room temperature and total lipid content was. It was then converted to Fatty Acid Methyl Esters (FAMEs).

2.5. Fatty Acid Methyl Esters (FAMEs)

FAMEs were extracted by following the method of White *et al.* (1998). Total lipid extracts were vortex-mixed for 30 sec in 2 mL of a mixture of methanolic KOH solution (0.2 mol L^{-1}) and toluene-methanol, 1:1 (v/v), incubated at 37ºC in a water bath for 15 min, cooled, and neutralized with acetic acid (1 mol L^{-1}). Two millilitres of hexane:chloroform, 4:1 (v/v) and 2 mL of Milli-Q water were added to the samples, centrifuged for 5 min at 2000 rpm to separate the phases. The upper phase was washed twice with 2 mL of hexane:chloroform, 4:1 (v/v). The supernatant obtained was mixed with the supernatant of the first wash, and then evaporated under room temperature and analysed by GC-MS.

3. Results and Discussion

3.1. Effect of sodium silicate

3.2. Biomass and lipid production

The alga grown at different concentrations of sodium silicate showed a typical growth curve i.e., Day 0 to Day 3 lag phase, Day 6 to Day 15 exponential phase and after Day 15th stationary phase. Among the seven different concentrations of sodium silicate chosen the alga showed

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maximum biomass of 266 mg L^{-1} at 0.245 mM, which was 175% more than control (Fig.1.). In various concentrations of sodium silicate, the lipid content extracted from the alga was calculated in term of percentage based on dry weight. The alga had high lipid accumulation of 43.12%, 42.21% and 41.60% at 0.07, 0.035 and 0.140 mM, respectively. The increments were 10-13% more than control (Fig.2). Lower concentrations of sodium silicate induced high lipid content (Lynn *et al.* 2000). Stationary phase may be induced by limiting one or more variables that control growth, such as nitrogen, phosphorus or silicon limitation (Mansour *et al.* 2005).

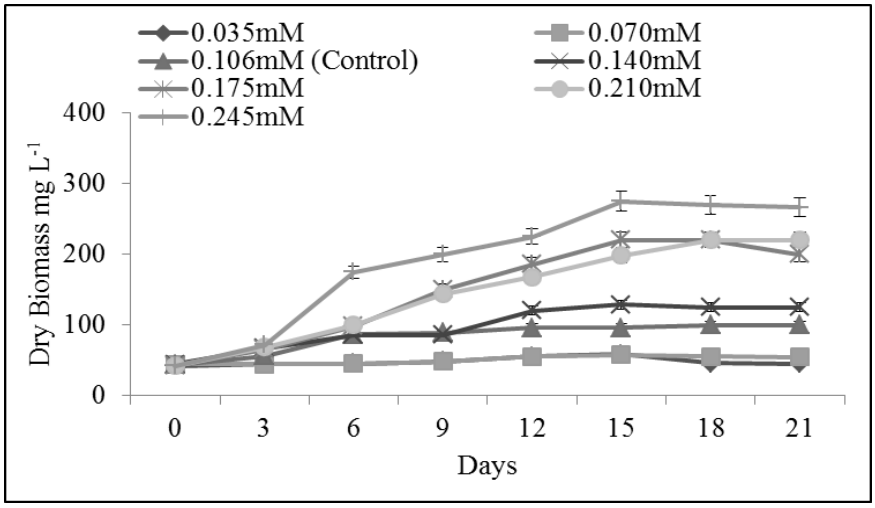

Fig.1. Effect of sodium silicate on biomass production of *A. paludosa*

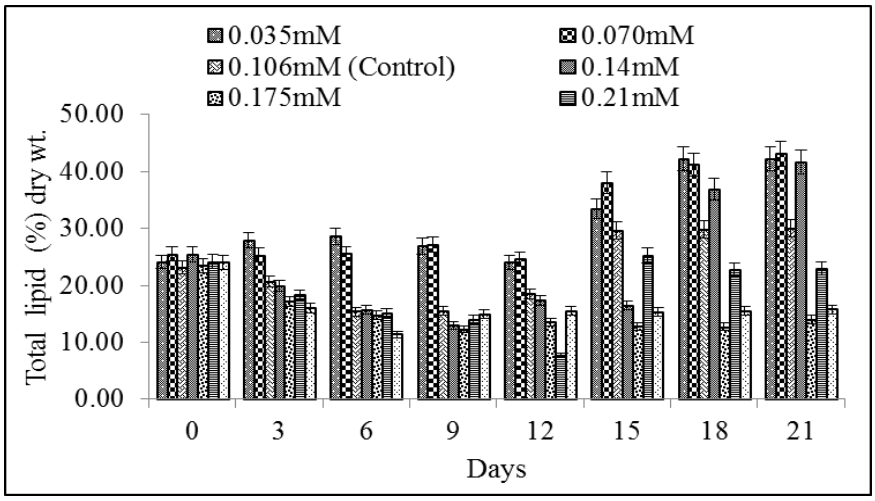

Fig.2. Effect of sodium silicate on total lipid content of *A. paludosa*

3.3. Effect of sodium nitrate

3.4. Biomass and lipid production

Among the different concentrations of sodium nitrate tested 0.94 and 1.0 mM supported maximum biomass production of each 180 mg L^{-1}, respectively. They were 12.5% more than control (Fig.3.). Nitrogen starvation has lead to lipid

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accumulation in a number of microalgal species *Neochloris oleoabundans* was able to accumulate 35-54% lipids under nitrogen-deficient conditions, of its cell dry weight and its TAGs comprised 80% of total lipids (Kawata *et al.*, 1998; Tornabene *et al.*, 1983). In *A. paludosa* maximum high lipid accumulations of 36.67% and 34.56% were recorded at 0.940 mM and 0.76 mM, respectively. Their increments were 6.67 and 4.56%, when compared to control (Fig.4.)

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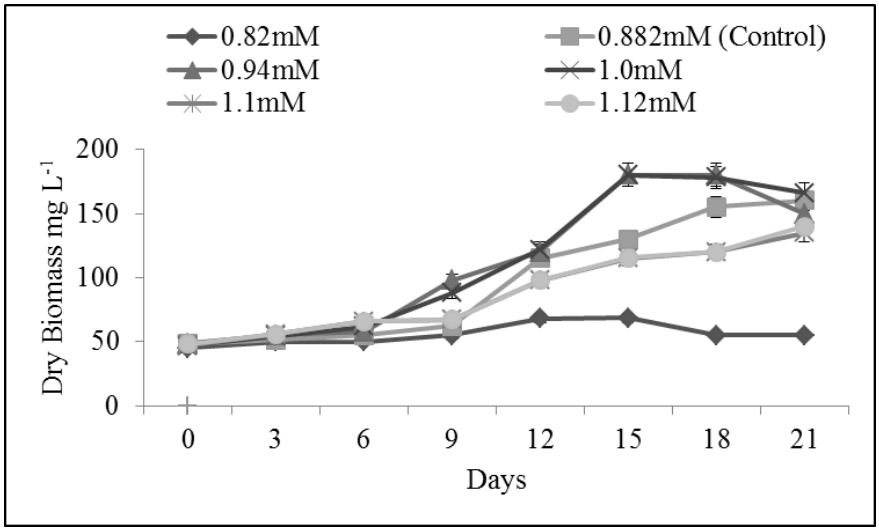

Fig.3. Effect of sodium nitrate on biomass production of *A. paludosa*

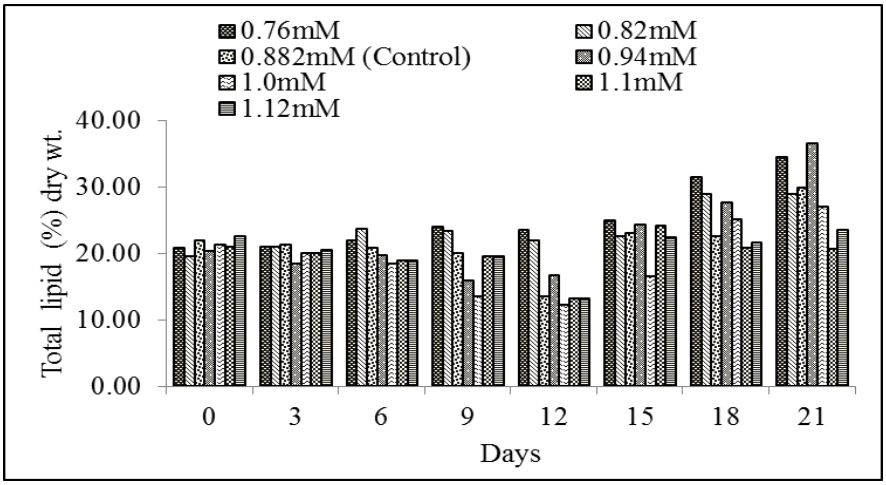

Fig.4. Effect of sodium nitrate on total lipid content of *A. paludosa*

3.5. Effect of Fe-EDTA

3.6. Biomass and lipid production

The alga showed high biomass in the medium amended with 0.026, 0.023 and 0.020 mM concentrations of Fe-EDTA. In the above conditions it showed 196.5 190.3 and 188.5 mg L^{-1} of biomass, respectively. Their increments were 46.5, 40.3 38.5% more than control (Fig.5.). The alga was able to accumulate high lipid content of 65.64%, 63.16% and 57.78% at 0.026, 0.023 and 0.009 mM, respectively. Their increments were 23.76, 21.29 and 15.91% more than control (Fig.6). Liu *et al.* (2008) reported similar values in *Chlorella vulgaris* were

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accumulated lipid content was 56.6% in iron deficiency condition.

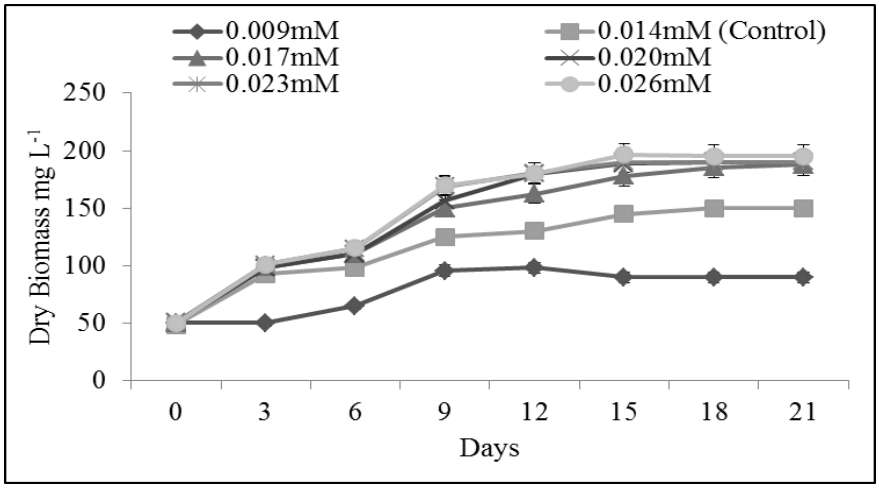

Fig.5. Effect of Fe-EDTA on biomass production of *A. paludosa*

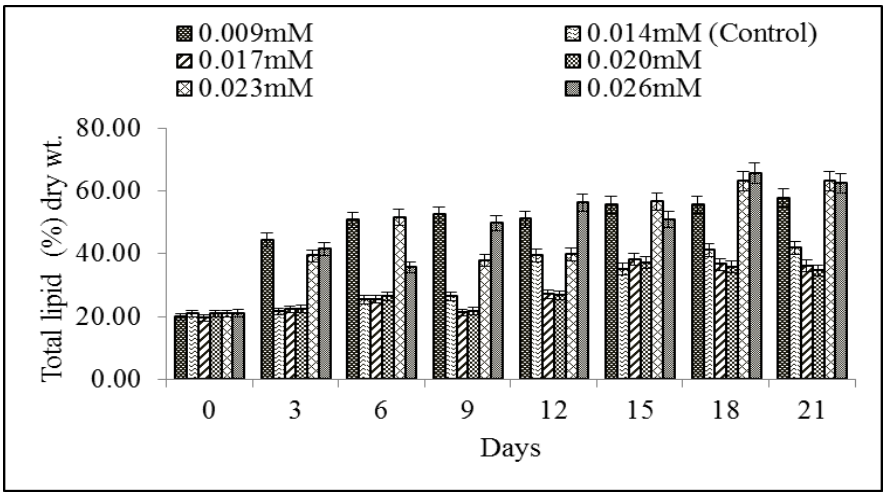

Fig.6. Effect of Fe-EDTA on total lipid content of *A. paludosa*

3.7. Effect of sodium phosphate

3.8. Biomass and lipid production

Amphiprora paludosa grown at different concentrations of sodium phosphate revealed that the following observations. High biomass production was observed at 0.075, 0.067 and 0.058 mM of 0.075, 0.067 and 0.058 mM on 21st day culture, respectively. Their increments were 5.0, 4.5 and 4.5 folds to that of control (Fig.7). The alga had high lipid 38.19% at 0.025mM which was 8.07% more than control (Fig.8). The deficiencies of a few nutrients, including phosphate (Khozin-Goldberg and Cohen 2006), have been reported as being able to cause the cessation of cell growth, and channel metabolic flux generated in photo biosynthesis to lipid/fatty acid biosynthesis.

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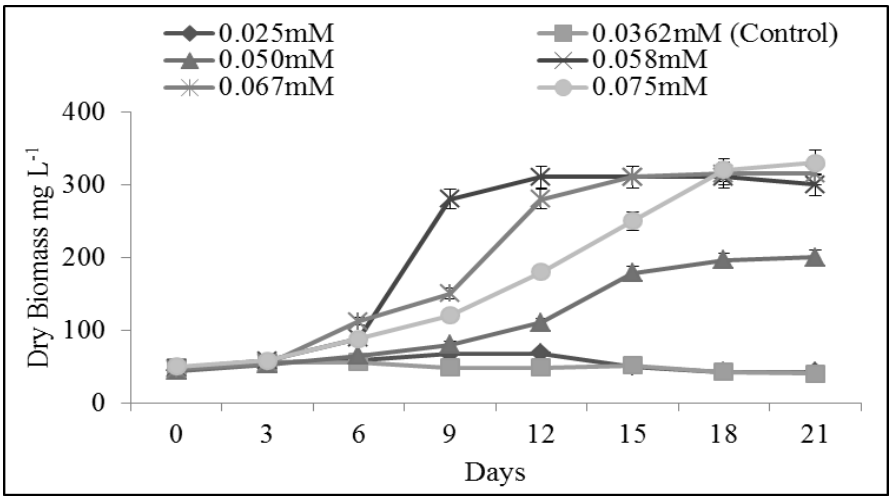

Fig.7. Effect of sodium phosphate on biomass production of *A. paludosa*

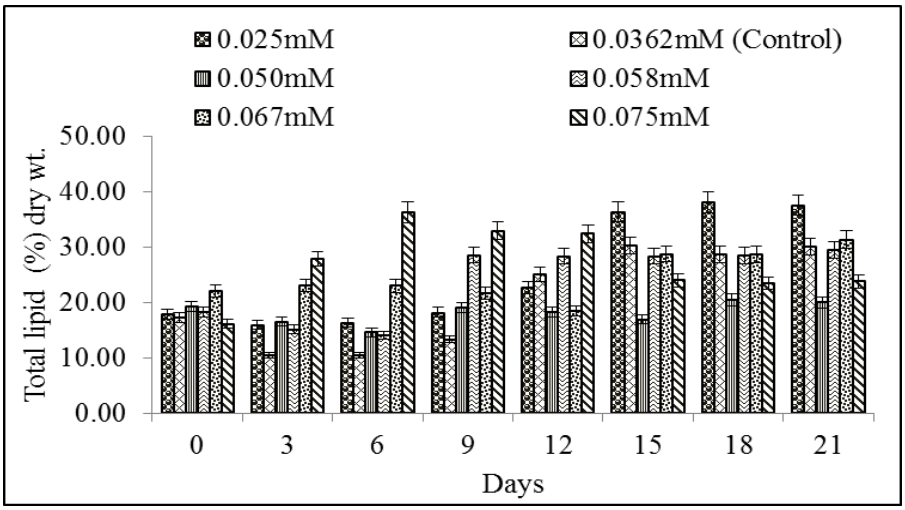

Fig.8. Effect of sodium phosphate on total lipid content of *A. paludosa*

3.9. Fatty acid composition

Amphiprora paludosa showed 17 different fatty acids: 6-Octadecenic acid 18:1 (n-7), 0.14%; 7-Octadecenoic acid 18:1, 0.32%; 7,10 Hexadecadienoic acid 16:2, 1.0%; Behenic acid 22:0, 0.37%; Lauric acid 12:0, 0.17%; Lignoceric acid 24:0, 0.54%; Linoleic acid 18:2 *n-6cis*, 0.47%; Margaric acid 17:0, 1.00%; Myristic acid 14:0, 6.08%; Nonanedioic acid 9:0, 0.11%; Oleic acid 18:1*n-9cis*, 2.19%; Oxiraneoctanoic acid 8:0, 0.56%; Palmitic acid 16:0, 52.62%; Palmitoleic acid 16:1, 26.23%; Pentadecanoic acid 15:0, 5.37%; Stearic acid 18:0, 2.76%; Tridecanoic acid 13:0, 0.1%. Among them 69.8% was saturated fatty acids, 28.88% of monounsaturated fatty acids and 1.47% of polyunsaturated. The fatty acids of diatoms have been studied more extensively than those of other microalgal groups (Reitan *et al.*, 1994; Zhukova and Aizdaicher, 1995; Grima *et al.*, 1996; Zhou *et al.*, 1996). It was reported that almost all diatoms contained high proportions of 14:0, 16:0; 16:1(n-7) and 20:5 (n-3) fatty acids, and C18 and C22 PUFAs were found as minor constituents (Orcutt and Patterson, 1975; Volkman *et al.*, 1989). Similar results were observed in the present study.

4. Conclusions

In this study, biomass and total lipid content of *Amphiprora paludosa* were evaluated at various concentrations of sodium silicate, sodium nitrate, Fe-EDTA and sodium phosphate in f/2 medium in the laboratory conditions. The biomass of the alga was enhanced when the medium amended with 0.075 mM of sodium phosphate. Whereas Fe-EDTA at 0.026mM supported maximum total lipid accumulation of the alga. It was suggested that among the four different nutrients chosen sodium phosphate and Fe-EDTA could play a vital role in biomass and lipid production.

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