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Environmental Stress Control to enhance lipid content in Oleaginous Microalgae for Biodiesel production

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Abstract: The aim of the present study is to evaluate the effect of nitrogen and phosphorus concentration in BG-11 medium to accumulate the higher amount of lipid content in the cells of oleaginous microalgae, which is a raw material for biodiesel production. The ability of *Chlorella vulgaris* growth was tested in the medium containing the presence of nitrogen and phosphorus (N+P+), medium with nitrate deficiency (N-P+), medium with phosphorous deficiency (N+P-) The achievement of high biomass concentration and abundant lipid accumulation are determined by performing dry weight determination and lipid accumulation experiments in the cells. The optimization of nitrogen and phosphorous was also achieved by performing the experiment for 12 days at different varying concentration of Sodium nitrate from 0g/L to 3.0g/L and Di- Potassium hydrogen phosphate from 0g/L to 0.5g/L in the presence of constant temperature 24± 2 ° C under 1500 $\mu mol^{-1} m^2 s^{-1}$ intensity with 10:14 hour's photoperiod.
Keywords: Biomass Concentration, Environmental stress, High Lipid content, oleaginous microalgae.

Introduction

The third generation fresh water oil accumulating microalgae, rich in lipids have the potential of biodiesel production **[2]**. The dominant, fast growing oleaginous microalgae produce large amount of algal biomass with low lipid content of about 5- 20% under optimal growth conditions. While the slow growing algal species produce algal biomass with high lipid content of about 20 -50% **[4]**. The microalgae biomass and Triacylglycerides (TAG) compete for photosynthetic assimilation and alteration of physiological pathways to stimulate lipid biosynthesis. The lipid metabolism of the fast growing microalgae can be enhanced further by subjecting the microalgae to an environmental stress that can alter the lipid biosynthetic pathway **[14]**. The saturated and unsaturated fatty acids synthesized with high nutritional value are produced by microalgae under optimal growth conditions, but are less ideal for biodiesel production **[3] [6]**. However, the synthesis of suitable precursor neutral lipids in the form of Triacylglycerides (TAG) can be enhanced under stress conditions favoring biodiesel production. The high lipid induction technique in oleaginous microorganisms can be studied by nutrient stress, Nitrogen and phosphorus starvation, varying pH, temperature, light irradiation, alterations of heavy metals and other chemicals. The present study will focus on the effect of Nitrogen and Phosphate limitation on high lipid induction under constant pH, temperature, and light irradiation.

Triacylglycerides (TAG) serve as energy storage in Microalgae and can be easily converted into biodiesel through transesterification reaction. Microalgae can be efficiently grown in non-arable land, utilize waste water and they do not compete with food crops for land and water. The oil production of microalgae is more than that of oil crops. *Chlorella vulgaris* is a familiar algae for bio-fuel production because of its fast growth and easy cultivable nature. The accumulation of lipids in microalgae is generally neutral lipids, polar lipids, wax esters, sterols, hydrocarbons, prenyl and pyrrole derivatives **[7]**. Polar lipids and non-polar lipids are the lipids produced by microalgae. The non-polar lipids are in the form of Triacylglycerides made of saturated fatty acids and some unsaturated fatty acids whereas polar lipids are rich in polyunsaturated fatty acids alone and act as a permeable barrier for cells and organelles. Triacylglycerides are mostly characterized by both saturated and monounsaturated fatty acids synthesized in the light, stored in the cytosolic lipid bodies, and then reutilized for polar lipid synthesis in the dark.

During the algal cultivation with required nutrient availability, photosynthesis is favored leading to the synthesis of ADP and NADPH molecules which in turn are taken up by the algal biomass. Continuous availability of required nutrients will favor photosynthesis leading to the formation of ADP and NADPH and utilization of the same by the biomass cyclically. Enhancement of the lipid content in the biomass is achieved by limiting the nutrients, thereby limiting the photosynthetic pathway, as the ADP and NADP+ molecules are available for Fatty Acid production instead **[5].**

Nitrogen and phosphate are the nutrients affecting lipid metabolism in algae. The deficiency of nitrogen and phosphate in the medium helps towards the accumulation of large amount of lipids in the form of Triacylglycerides has been observed in various strains of microalgae cultivated in a low Nitrogen and Phosphate medium. It is observed that the nitrogen and phosphate depletion in the medium changes the lipid nature from free fatty acid rich lipids to lipids mostly contained as triacylglycerides. It is also observed that the nutrient starvation in microalgae not only affects the fatty acids metabolism, but also affects the pigment composition.

Material and Methods

The Micro algal samples were collected from stagnant fresh water bodies in Pallikaranai, Chennai, India. Initially the samples were enriched and maintained in BG-11 broth in 250 ml conical flasks at 24± 2 °C under 1500 $\mu mol^{-1} m^2 s^{-1}$ intensity with 10:14 hour's photoperiod and shaken manually for three to four times a day in order to prevent the stickiness of the algae and to introduce aeration to the culture samples. The fresh nutrient medium with varying concentrations of Nitrogen and Phosphate were prepared and cultured with *Chlorella vulgaris* and monitored for the determination of dry weight and total lipid content at frequent intervals **[12] [10]**. The dry weight was determined by filtering the algal culture onto pre-weighted Grade 1 Qualitative Whatman filter paper containing 11µm pore size ; diameter 9.0 cm and washed with distilled water. Then the cells on the Qualitative Whatman filter paper discs were dried in hot air oven at 60°C until the weight remains constant. Total lipid measurements were performed by following the widely practiced Bligh and Dyer method **[1-]** by drying in a hot air oven at 65 ^{o}C. 10 ml of algal culture samples were collected and centrifuged at 4000 rpm for 5 mins. To the cells 2 ml chloroform, 4 ml methanol and 1.6 ml Distilled water were added and the samples were kept in dark for 40 hrs under room temperature. The sample mixtures were then centrifuged at 4000 rpm for 5 mins and the supernatant were collected to pre weighed glass tube (M1 g). Then, 2 ml methanol and 1 ml chloroform were added to the sample mixture and kept in dark again for 24 hrs under room temperature **[9]**. The sample mixtures were again centrifuged at 4000 rpm for 5 mins and the extracts were collected into pre weighed glass tube. Now, 3 ml chloroform and 3.8 ml distilled water were added to the mixture sample for the phase separation where, and the bottom layer was air dried in hot air oven at 65°C until the weight was constant (M2 g). Lipid content was calculated by subtracting M2 and M1 **[13] [11] [8]**.

Results and Discussion

Nitrogen and Phosphorus deficiency as Environmental stress controlled for Enhanced lipid content in *Chlorella vulgaris*

The *Chlorella vulgaris* was inoculated with the initial dry weight of about 0.9 g/L in Nitrate and phosphorus deficient medium and studied for its high biomass concentration and abundant lipid accumulation for 12 days. The highest biomass concentration of about 6.5 g/L was observed at the end 12th day in the medium containing both nitrate and phosphorus, whereas the maximum biomass concentration in Nitrate deficiency medium was 5.2 g/L was obtained. The least biomass concentration was observed to be 4.3 g/L shown in **(Fig.1)**. Though the biomass concentration was seen to be less in nitrate deficiency medium the cells grown in nitrate deficient medium accumulates 49.2% of lipid content when compared to phosphate deficient medium of about 45.3 % and simultaneously the medium with nitrate and phosphorus accumulates lipid of about 38.3% . Thus Nitrate deficient medium seems to be an efficient medium for abundant lipid accumulation in *Chlorella vulgaris* ***(Fig.2)***.

Effect of Nitrogen and Phosphorus Concentration on the growth of *Chlorella vulgaris*

The effect of nitrogen and phosphorus at different concentration was studied for 12 days for its high biomass concentration in *Chlorella vulgaris* (Table.1) at constant temperature of 24± 2 ° C under 1500 µmol-1m2s-1 intensity with 10:14 hour's photoperiod **(Fig.3)**. The medium containing 1.5 g/L of nitrate and 0.2 g/L of Di-potassium hydrogen phosphate results in occurrence of high biomass production when compared to other medium concentrations. **(Fig.4)**

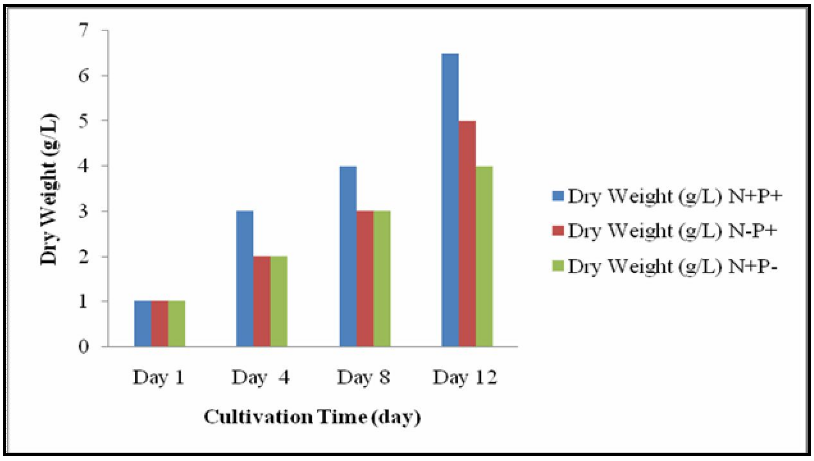

Fig.1 Effect of Nitrogen and Phosphorus Concentration on the growth of *Chlorella vulgaris*

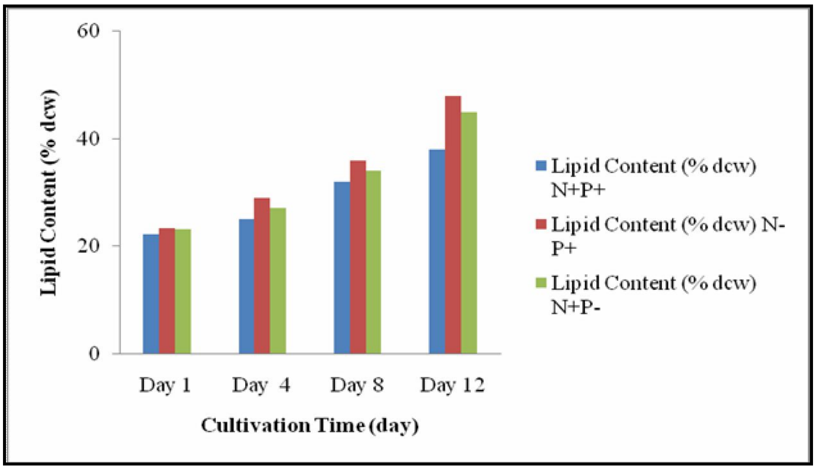

Fig.2 Nitrogen and Phosphorus deficiency as Environmental stress controlled for Enhanced lipid content in *Chlorella vulgaris*

Table. 1 Different Concentrations of Nitrogen and Phosphorus used for the growth of *Chlorella vulgaris*

Composition	Medium -1 (M1) g/L	Medium -2 (M2) g/L	Medium – 3 (M3) g/L	Medium – 4 (M4) g/L	Medium -5 (M5) g/L
Sodium nitrate (NaNo3)	0	0.1	1.0	1.5	3.0
Di-Potassium Hydrogen Phosphate (K2HPO4)	0	0.01	0.04	0.2	0.5

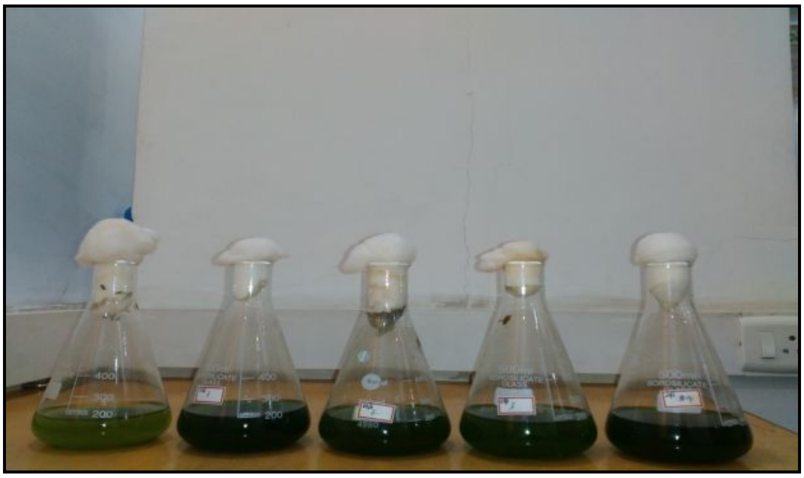

Fig.3 Culturing of *Chlorella vulgaris* at varying concentration of Sodim Nitrate and Di- potassium hydrogen phosphate

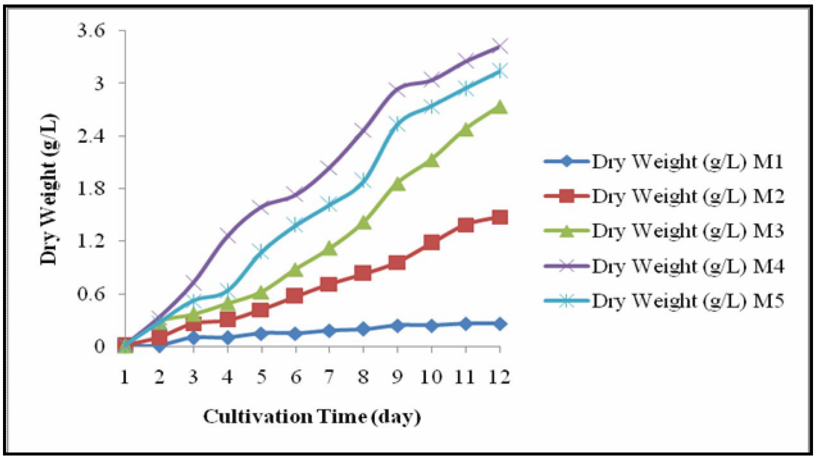

Fig.4 Growth of *Chlorella vulgaris* under different Nitrogen and Phosphate Concentration

V. Conclusion

The present study concludes that *Chlorella vulgaris* has the ability to accumulate abundant amount of lipid in the nitrate deficiency medium and achieve its high biomass concentration at the presence of both nitrogen and phosphorus in the BG-11 medium. Thus the different concentration of sodium nitrate and di-potassium hydrogen phosphate are optimized for the growth of *Chlorella vulgaris* to accumulate high lipid content and high biomass production has been achieved. Thus by inducing the nutrient stress condition is studied not only for the lipid accumulation but also for its microalgae growth reduction.

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