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## **Effect of Salinity Concentrations on Growth Rate and Lipid Concentration in *Microcystis* Sp., *Chlorococcum* Sp. and *Chaetoceros* Sp.**

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Biofuels from microalgae is a viable alternative for replacing the global demand for petro-diesel. The two important desirable characteristics considered in a species to be used for biodiesel production are high biomass and lipid production. It is noted that the increase in salinity can increase the lipid content of microalgae, but lowers the growth rate of a species. Therefore, the effect of salinity on the growth and lipid content of microalgal species have to be investigated. Salt stress is a major abiotic environmental factor that limits plant growth and productivity. The salinity stress and unfavorable light conditions are the main limiting factors of plant productivity both in aquatic and terrestrial, natural and anthropically modified environments. Microalgae differ in their adaptability to salinity and other stress conditions. The ability of cells to survive and flourish in saline environment under the influence of osmotic stress has received considerable attention. Under favorable and unlimited growth conditions microalgae produce primarily polar lipids (e.g. glycolipids and phospholipids), which enrich chloroplast and cellular membranes. However, under unfavorable growth conditions microalgae accumulate neutral lipids in lipid droplets located in the cytoplasm. The study focuses on the effect of salinity concentrations on the cell growth and lipid content of three microalgae viz., *Chlorococcum* sp., *Microcystis* sp. (fresh water algae) and *Chaetoceros* sp. (marine alga), isolated and cultured in appropriate medium for a period of 7 days.

The halotolerance of all the three algae were determined by growing them in three different salinity concentrations. All the three microalgae were able to tolerate the salinity levels and showed different growth patterns and lipid accumulation rates. The cell growth of all the three algae did not show a definite pattern. The total lipid content was found to be higher on the 5th day of culture experiment in case of *Chaetoceros* sp (8.06 mg/ml at 35 ppt) and *Microcystis* sp. (8.4 mg/ml at 0.2 ppt) whereas, it was higher on 6th day for *Chlorococcum* sp (6.6 mg/l at 0.2 ppt). The increase in lipid content at higher NaCl concentration may be due to adaptation under stress conditions, which help in accumulation of lipid content in cells.

Keywords: Microalgae, NaCl, Lipid, *Chlorococcum*, *Microcystis*, *Chaetoceros*.

## 1. INTRODUCTION

Algal biomass can serve as a feedstock for the production of a variety of different biofuels, e.g. biodiesel, hydrogen, methane and bioethanol. Biodiesel is a non-toxic and biodegradable alternative fuel derived from renewable sources (Hussain et al., 2008). Microalgal biofuel are derived from the lipid content of the algal cells that serve as the feedstock for many high energy density transportation fuels, including biodiesel as well as green diesel, green jet fuel and green gasoline (Pienkos, 2009). The lipid and fatty acid composition of microalgae differs according to the culture conditions. The lipid accumulation in algae usually occurs during environmental stress, including growth under nutrient deficient conditions. The cells adapt themselves to stress by undergoing changes in morphological and developmental pattern as well as physiological and biochemical processes. The increase in salt concentration affects the rate of respiration, distribution of minerals, ion toxicity, photosynthetic rate and permeability of the cell membranes (Sudhir, 2004). Some unicellular green alga like *Dunaliella salina* var. *bardawil* responded to salinity stress by regulating carbon fluxes between starch production in the chloroplast and the synthesis of glycerol in the cytoplasm (Cowan, 1991). The growth of microalgae is retarded during salinity stress due to the accumulation of compatible solutes like proline and glycine to balance the external salt concentrations (Ahmed et al, 1989). Compatible

solutes like proline are formed within cells during osmotic stress and acts as osmoprotectants to stabilize enzymes (Fatma et al, 2007). The percentage of saturated fatty acid in microalgae decreased as the concentration of NaCl increased, while the percentage of highly unsaturated fatty acid increased (Kirrolia et al, 2011). The decrease in photosynthetic activity commonly observed under salt stress may be due to limitations in photosynthetic electron transport and partial stomatal closure (Zhang et al, 2010). In *Synechocystis*, it has been found that the combination of light and salt stress has a strong synergistic and damaging effect on PSII, which is due to the fact that salt stress inhibits the activity of PS II from light-induced inactivation (Allakhverdiev et al, 2002).

## 2. OBJECTIVES

The main objective of this study is to investigate the effects of various salinity conditions on the cell growth and lipid production of three microalgae. This involved

- a. Isolation of *Chlorococcum* sp., *Microcystis* sp. and *Chaetoceros* sp. from natural conditions
- b. Monitoring the cell growth in different salinity concentrations
- c. Analysis of the variation in cell growth and lipid concentration in microalgae.

### 3. MATERIALS AND METHODS

#### 3.1. Microorganisms and Culture Conditions

The freshwater algae used in the present study were collected using plankton net from the Centenary pond, Indian Institute of Science, Bangalore. *Chaetoceros* sp. was isolated from a marine sample and cultured. The medium used to culture microalgae viz., *Chlorococcum* sp. and *Microcystis* sp. was Bold's basal media (Bold 1949, Bischoff and Bold 1963) and for *Chaetoceros* sp. was f/2 media (Guillard and Ryther, 1963). Initial stock cultures of *Chlorococcum* sp., *Microcystis* sp. were maintained in BBM and *Chaetoceros* sp. was cultured in f/2 media and incubated for 10 days at 25°C under 16:8 light:dark (L:D) photoperiod in culture room. The halotolerance was determined using different NaCl concentrations i.e., 0.2 ppt, 1 ppt and 2 ppt for freshwater algae and 35 ppt, 175 ppt and 350 ppt for marine alga. To study the effect of salinity on the three different algae, experiments were carried out in 250 ml Erlenmeyer flasks each containing 100 ml of appropriate medium incubated at 25°C under 16:8 light : dark (L:D) photoperiod. Control cultures were also maintained under similar culture conditions. The experiments were set up for 7 days. The cell count and extraction of total lipid of all the three microalgae at three different salinities were determined every day. All the experiments were carried out in duplicates.

#### 3.2. Extraction of Total Lipids

The total lipids were extracted by mixing chloroform – methanol (4:2 v/v) with the algal samples using slightly modified version of Bligh and Dyer's method (Bligh and Dyer, 1959). Algal biomass was collected by centrifuging the algal culture at 3000 rpm for 10 minutes. The

supernatant was discarded in case of *Chlorococcum* sp. and *Chaetoceros* sp. whereas cells of *Microcystis* sp. were collected from the upper part of the centrifuge tube. The algal biomass was suspended in 4 ml chloroform and 2 ml methanol and shaken well. The cells were then, subjected to sonication for the complete disruption of cells for 1 hour. The chloroform – methanol forms a biphasic layer. The lower lipid layer was separated carefully using the eppendorf micropipette and transferred into a centrifuge tube. About 2 ml of distilled water was added and vortex well for further purification. The total lipid was transferred to clean dried and weighed glass centrifuge tube. The weight of total lipid was determined gravimetrically.

### 4. RESULTS AND DISCUSSION

#### 4.1 The Effect of Salinity on the Growth Rate of Microalgae

The three microalgae viz., *Chlorococcum* sp., *Microcystis* sp. and *Chaetoceros* sp., were isolated and cultured in BBM media, BBM media and f/2 media respectively for a period of 7 days. Varying salinity levels were given to check for the difference in cell growth in each of the treatments.

***Chlorococcum* sp.:** As seen in figure 1, in case of 0.2 ppt, the cell growth doubled ( $12404 \times 10^2$  cells/ml) on 4<sup>th</sup> day and hence after a sudden decrease in the cell growth was recorded. For the control i.e., for 1 ppt, the cell growth was found to be maximum on the 2nd day ( $9828 \times 10^2$  cells/ml), a slight decrease was seen on 3<sup>rd</sup> day. The cell count also decreased from 5<sup>th</sup> day like the first treatment. For 2 ppt, the cells showed an increase on 6<sup>th</sup> day ( $12638 \times 10^2$  cells/ml).

***Microcystis* sp.:** As seen in Figure 1, in case of 0.2 ppt, the cell growth is seen to be highest on 3<sup>rd</sup> day i.e.,  $1824 \times 10^2$  cells/ml and the lowest was seen on 7<sup>th</sup> day with  $200 \times 10^2$  cells/ml. For control i.e., 1 ppt, maximum cell growth of  $1172 \times 10^2$  cells/ml and minimum of  $56 \times 10^2$  cells/ml was recorded on 2<sup>nd</sup> and 7<sup>th</sup> day respectively. A similar trend was found in the treatment with 2 ppt, maximum was found on 2nd day ( $1875.5 \times 10^2$  cells/ml) and minimum being  $264 \times 10^2$  cells/ml on 7<sup>th</sup> day.

### 3.2 Effect of Different Salinity Levels on Total Lipid Concentration:

***Chaetoceros* sp.:** 3rd day of 35 ppt (control) treatment, the cell growth was found to be maximum with  $2490 \times 10^2$  cells/ml while minimum being on the 6th day i.e.,  $444 \times 10^2$  cells/ml. The growth rate was found to be maximum on the 5th day for the treatment with 175 ppt while 6th day being the lowest. 350 ppt showed different response to the cell growth of *Chaetoceros* sp. with least being on 2nd day and highest on the 3rd day (Figure 1).

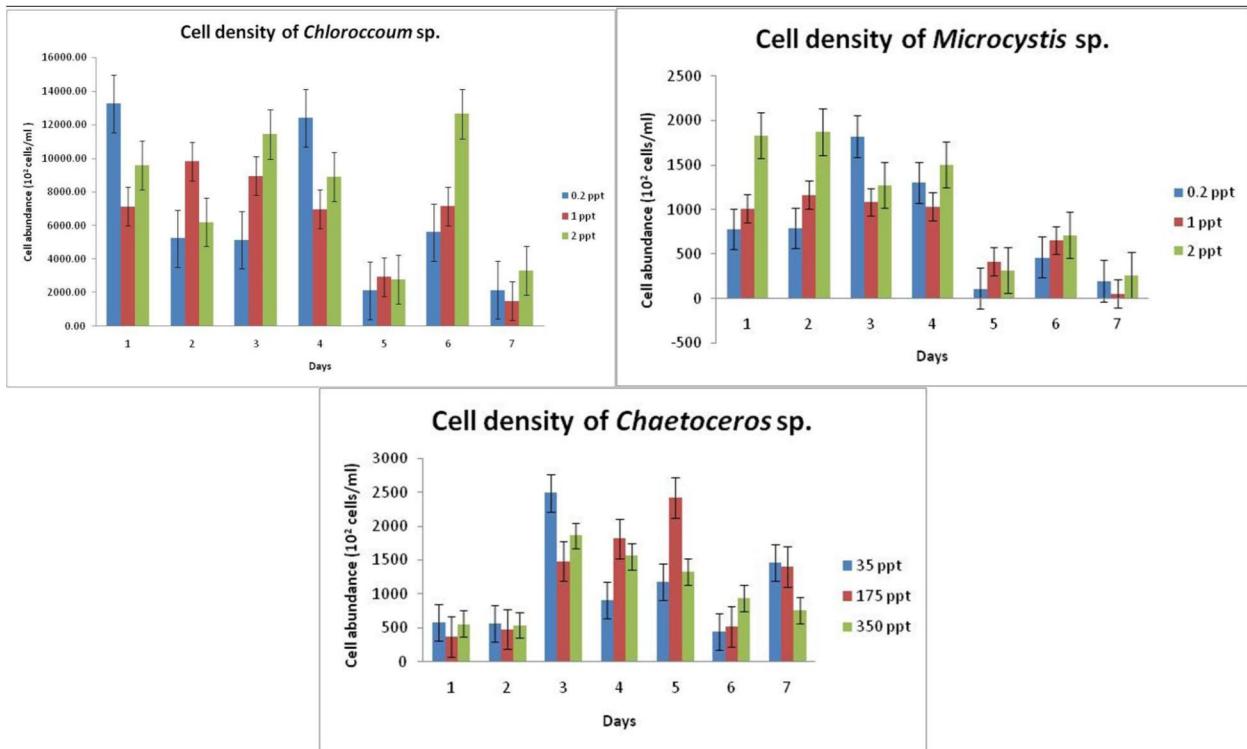


Figure 1: Cell density of *Chlorococcum* sp., *Microcystis* sp. and *Chaetoceros* sp. with response to different salinity concentrations

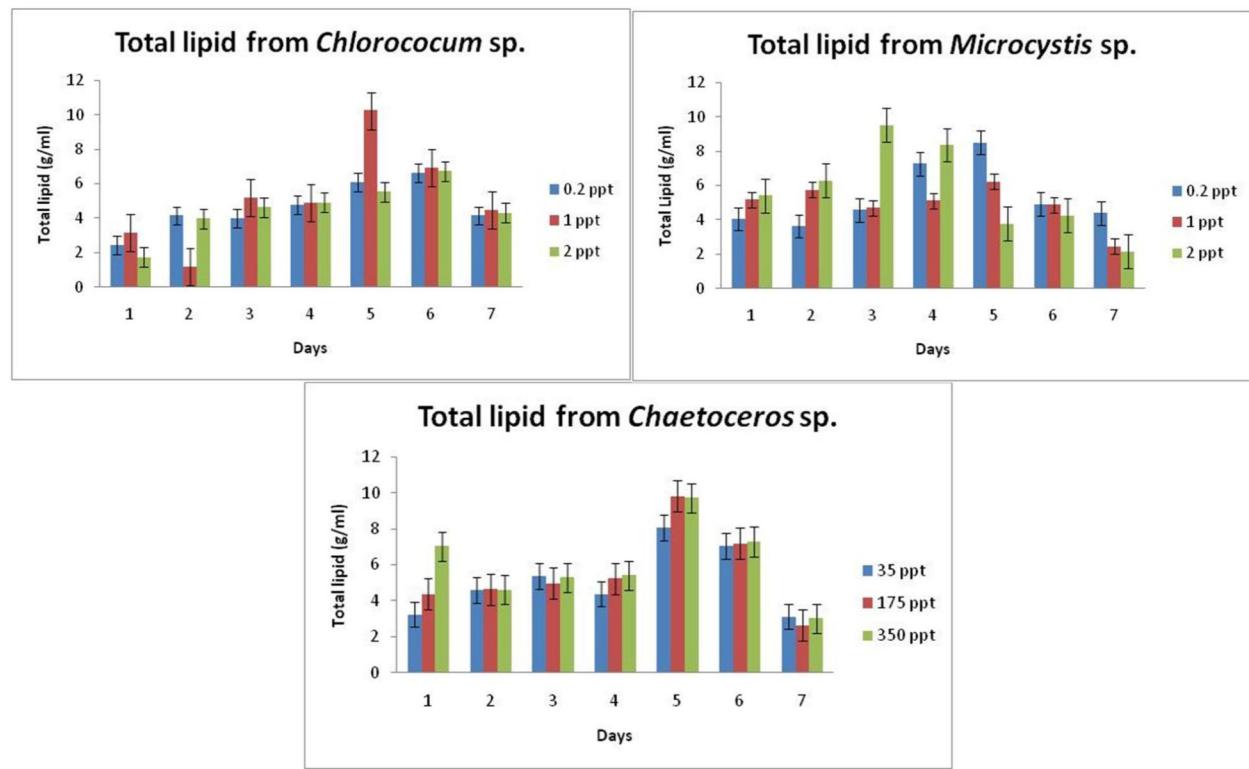


Figure 2: Total lipid of *Chloroccum* sp., *Microcystis* sp. and *Chaetoceros* sp. with response to different salinity concentrations

***Chlorococcum* sp.:** As seen in figure 2, in case of 0.2 ppt, total lipid is higher (6.6 mg/l) on 6th day with low lipid content recorded on the 1st day. For the control i.e., for 1 ppt, the total lipid content was found to be maximum on the 5th day (10.24 mg/ml) with lowest total lipid content recorded on the 2nd day. For 2 ppt, the cells showed an increase on 6th day (6.7 mg/ml).

***Microcystis* sp.:** As seen in Figure 2, in case of 0.2 ppt, the lipid content is seen to be highest on 5th day i.e., 8.4 mg/ml and the lowest was seen on 2nd day with 3.6 mg/ml. For control i.e., 1 ppt, maximum lipid content of 6.2 mg/ml and minimum of 2.45 mg/ml was recorded on 5th and 7th day respectively. A similar trend was found in the treatment with 2 ppt, maximum was found on 3rd day (9.5 mg/ml) and minimum being 2.15 mg/ml on 7th day.

***Chaetoceros* sp.:** 5th day of 35 ppt (control) treatment the lipid content was found to be

maximum with 8.06 mg/ml while minimum being on the 7th day i.e., 3.12 mg/ml. The lipid content was found to be maximum on the 5th day of treatment with 175 ppt while 7th day being the lowest. 350 ppt showed different responses to the lipid content of *Chaetoceros* sp. with least being on 7th day and highest on the 5th day (Figure 2).

Although many species of micro-algae are tolerant to great variations in salinity, their chemical composition can be affected (Brown et al., 1989; Roessler, 1990). The difference in the growth response within the treatments suggests the existence of population genetic variation in the ability to cope with extremely low salinity and corroborates genetic and physiological heterogeneity. According to Cooksey and Chansang (1976), and Fabregas *et al.*, (1984) salinity stress triggers the production of lipids. Renaud *et al.* (1994) reported a 24.2 % total lipid content for a marine *Navicula* species. Three

freshwater *Navicula* species attained an average lipid content of 24 to 51 % of dry weight (DW) (Griffiths and Harrison, 2009), for nutrient replete and deficient conditions. Algae differ in their adaptability to salinity and based on their tolerance extent, they are grouped as halophilic (salt requiring for optimum growth) and halotolerant (having response mechanism that permits their existence in saline medium).

In either case, the algae produce some metabolites to adapt to salt injury and also to balance as per the surroundings osmotica (Richmond, 1986). Depending on the species under study, there may be an increase in ash content and total lipid content (Ben-Amotz et al., 1985). A similar salinity tolerance has been reported previously for *Isochrysis* sp. (Ben-Amotz et al., 1985; Fabregas et al., 1985; Jeffrey et al., 1990) and a range of other species (Jeffrey et al., 1990). An increase in fatty acid with increasing salinity has been reported previously for *Navicula oculata* (Hodgson, 1991). Many reports have suggested that lipids might be involved in the protection against salt stress (Huflejt et al. 1990, Khamutov et al. 1990, Ritter and Yopp 1993). When photosynthetic organisms are exposed to salt stress, the fatty acids of membrane lipids are desaturated. Hence in the given study, the increase in the salinity is perceived as an unfavorable condition resulting in a rise in the total lipid content.

## CONCLUSION

Salt stress significantly affects cells growth and lipid formation. Lipid act as a secondary metabolite for algae, and stress or unfavourable condition such as nutrients, growth phase, temperature, light, etc., leads to the accumulation of more lipids (Hu et al., 2008). Increase in the salinity impaired algal growth. As the increase or decrease of salinity generated stress inside the algae, total lipid content increased acting as a

storage reserve energy material till favorable conditions arise.

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