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Effect of Organic Carbon Compounds on the Growth and Pigment Composition of Microalga - *Nannochloropsis salina*

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Abstract

Heterotrophic and mixotrophic cultivations of microalgae produce better biomass and valuable chemicals through biorefinery processes. Present study analyses the effect of organic carbon sources such as glucose, fructose, lactose and sucrose on the growth and pigment composition of the microalga, *N.salina.* Among the different organic carbon sources tested*, N. salina* could grow as a mixotroph with the addition of low concentrations of fructose and sucrose, which produced better growth rates than under photoautotrophic conditions.

Keywords: *Nannochloropsis salina*, Microalgae, mixotrophy

INTRODUCTION

Culturing of microalgae has been carried out throughout the world in order to produce aquaculture feed, high value added products and biofuel. These organisms are usually cultivated in open or closed photo-bioreactors using CO_2 as a carbon source. However, these culture methods have several disadvantages including low cell densities and long cultivation time. Hence, mixotrophy or heterotrophy are feasible alternatives[1]. Usually algae are photoautotrophic organisms but some members could take up organic compounds from the medium as carbon source. This type of nutrition is called mixotrophy and it is defined as the capacity of an organism to be autotrophic and heterotrophic at the same time. This phenomenon was discovered during studies

on ciliates and flagellates present in the ocean[2]. Mixotrophy is prevalent in planktonic Chrysophytes, Prymesiophytes etc.[3]Due to its relatively lower requirement for light intensities, mixotrophic cultures produce better biomass and lipid in low cost medium[4]. Mixotrophic production of microalgal biomass has been demonstrated to be a viable alternative for the conventional photoautotrophic mass culture.

Many microalgae are able to take up organic compounds from the medium. Glucose, fructose, and glycerol can be taken up by a number of green microalgae[5]. Requirement of low light intensity and low energy cost make mixotrophy more popular in algal culture. The synergic effect of light and the organic carbon induces high productivity in mixotrophic cultures[6,7,]. Wong and Lay [8] used aqueous extracts of soyabean and tea leaves for the cultivation of *Chlorella pyrenoidosa*. Independent of the light and dark condition, *Chlorella vulgaris* and *Scenedesmus* sp. utilized organic substrates[9.] Tanner[10] reported that an inducible active hexose transport system in *C. vulgaris* and *C. protothecoides* can utilize glucose, acetate and other organic components in heterotrophic condition. Diatoms such as *Navicula saprophila*[11] and *Nitzschia* sp.[11,12,13]are also able to grow in mixotrophic conditions. The optimum condition for culturing *Scenedesmus* sp. AARL GO22, a promising organism for biofuel production is under mixotrophic culture [14]. The effect of carbon source on the growth and biochemical composition of microalgae has been reported for several species [15,12,16]. *Micractinium pusillum* could utilize glucose both in heterotrophic and mixotrophic condition[17]. Mixotrophic production of marine microalga, *Phaeodactylum tricornutum* on various carbon sources was studied[16]. For glycerol, fructose and glucose the biomass production was respectively 10, 8 and 5 fold higher than those obtained in control photoautotrophic cultures. Liu *et al.* [18] studied the effect of organic carbon source on the growth, photosynthesis and respiration of *Phaeodactylum tricornutum* and observed that 100 mM glycerol, acetate, and glucose significantly increased the specific growth rate, and higher biomass concentration was achieved in mixotrophic growth. Xin *et al.* [19] cultured *Scenedesmus* sp. in different carbon sources such as glucose, glycerol and sodium acetate and found glucose to be the most suitable organic carbon source for biomass production. Abreu *et al.* [20] reported mixotrophic cultivation of *Chlorella vulgaris* using byproducts of dairy industry. They suggested it as an alternative to reduce the cost of microalgal biomass production, since it does not require the addition of expensive carbohydrates. Hamed and Kl [21] reported good growth of green and blue-green algae on hydrolyzed molasses, a byproduct of sugar refinery. It is a cheap carbon source for mixotrophic cultures. Sharma *et al.* [22] studied the influence of organic carbon sources like sucrose, glycerol and glucose on the growth and lipid production in *Chlorella* sp. and found glucose to be the best organic carbon source to increase its biomass and lipid content. Velu *et al.* [23] studied the effect of carbon sources such as glucose, fructose, sucrose, lactose and galactose on biochemical production in marine microalgae, *N. salina, Dunaliella tertiolecta* and *Tetraselmis suecica* and found that the biomass yield and

lipid contents considerably increased with sucrose and fructose in comparison to that of control.

The present study aims to analyse the effect of organic carbon sources such as glucose, fructose, lactose and sucrose on the growth and pigment composition of the microalga, *N. salina*.

MATERIALS AND METHODS

Microalga, *N. salina* was obtained from the culture collection of Marine Botany Laboratory, Dept. of Marine Biology, Cochin University of Science and Technology. Glucose, fructose, lactose and sucrose were used as organic carbon sources in the experiments. The following concentrations were used; 0.5, 1.0 and 1.5 mg/L. Experiments were set up using 250 mL Erlenmeyer flasks containing 150 mL f/2 medium appended with the respective growth regulator/carbon source. 10% volume of logarithmically growing culture was used as inoculum. Illumination was provided by cold white fluorescent light of 2000 lux for a light/dark period of 12:12 hours, with a temperature range of 28-30°C. The culture was maintained for 12 days and the readings were taken at 2 day intervals. Growth rate was calculated using Guillard's equation [24].

$$r = \frac{\ln N_t - \ln N_0}{\Delta t}$$

Where r = exponential growth rate

$\ln N_t$ = population size at the end of the time interval

$\ln N_0$ = population size at the beginning of the time interval

Δt = length of the time interval.

Growth rate was expressed as growth/day.

Pigments estimations were done using the methods of Jeffrey and Humphrey[25]; Strickland and Parsons, [26].

Statistical analysis was done using one way analysis of variance (ANOVA) with SPSS version 17. The values were compared using Tukeys Test ($p< 0.05$).

RESULTS

Many microalgae are able to grow mixotrophically by combining photosynthesis with heterotrophic nutrition. In the present study *N. salina* was able to grow mixotrophically in some of the selected carbon sources.

Effect of glucose, as a carbon source on the growth rate of *N. salina* is presented in

Fig.1. A slight increase in growth rate was noticed in cultures with 1.0 mg/L glucose compared with that of control. Lowest growth rate was noted in cultures with 0.5 mg/L glucose (0.28/day). Statistical analysis showed that there is no significant difference between growth rates of *N. salina* in different glucose levels ($p = 0.152$, $p>0.05$). Chl *a* production decreased with increasing concentrations of glucose in the culture media (Fig.2). Cultures with 1.0 mg/L glucose produced the lowest amount of Chl *a* (186.57 µg/L) while cultures with 0.5 and 1.5 mg/L glucose produced 251.9 and 248.5 µg/L Chl *a* compared with 1281 µg/L in control. Production of total carotenoid was also low in cultures with glucose as carbon source (Fig.3). Lowest production of carotenoids was noted in cultures with 0.5 mg/L glucose (320 µg/L). Medium containing 1.0 mg/L and 1.5 mg/L glucose produced only 346.7 and 333 µg/L carotenoid respectively. The control produced 620 µg/L of total carotenoid.

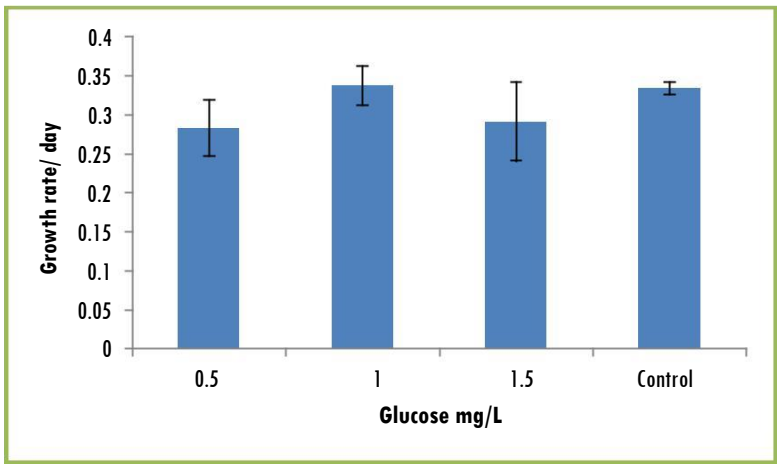

Fig.1 Effect of glucose on the growth rate of*N. salina*

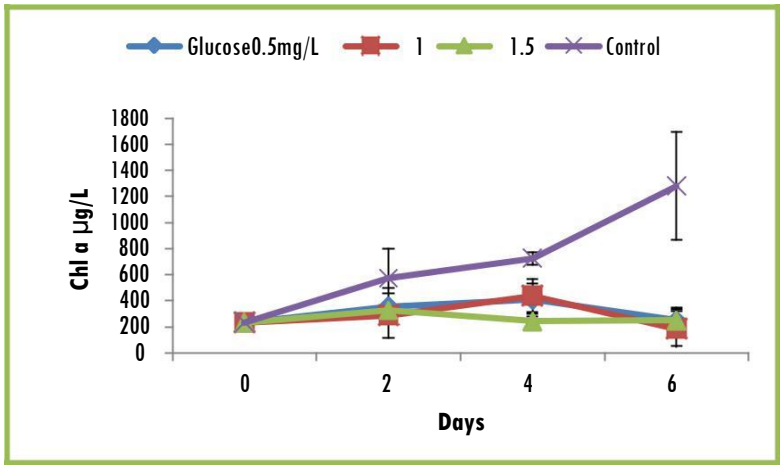

Fig.2 Effect of glucose on Chl*a* production in *N. salina*

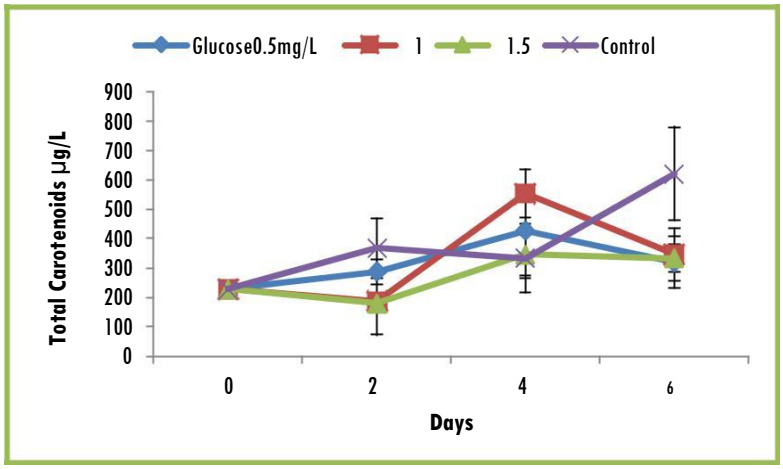

Fig.3 Effect of glucose on total carotenoid production in *N. salina*

Lower concentrations of fructose had a significant positive effect on the growth rate of *N. salina* (Fig.4). However, increasing concentrations of fructose adversely affected the growth rate. Growth rate of *N. salina* culture with 0.5 mg/L fructose as carbon source was noted as 0.3495/ day, whereas, in the control it was 0.334/ day. The lowest growth rate was noted in 1.5 mg/L fructose added culture (0.256/ day). ANOVA results showed that there is significant difference between growth rates of *N.salina* in different fructose levels ($p = 0.015$, $p<0.05$). Production of Chl *a* decreased with the addition of fructose as carbon source in the medium (Fig.5). Lowest amount of Chl *a* was produced in cultures with 1.5 mg/L fructose (337.05 µg/L), whereas in cultures with 1.0 mg/L fructose the production was 513 µg/L. Control produced 1281 µg/L Chl *a*. Comparatively high amount of carotenoid was produced in cultures with 1.0 mg/L fructose (Fig.6). Total carotenoid produced by this culture was 660 µg/L, compared with 620 µg/L carotenoid in the control. Production of carotenoid was low in cultures with 0.5 and 1.5 mg/L fructose, 400 µg/L and 413.3 µg/L respectively.

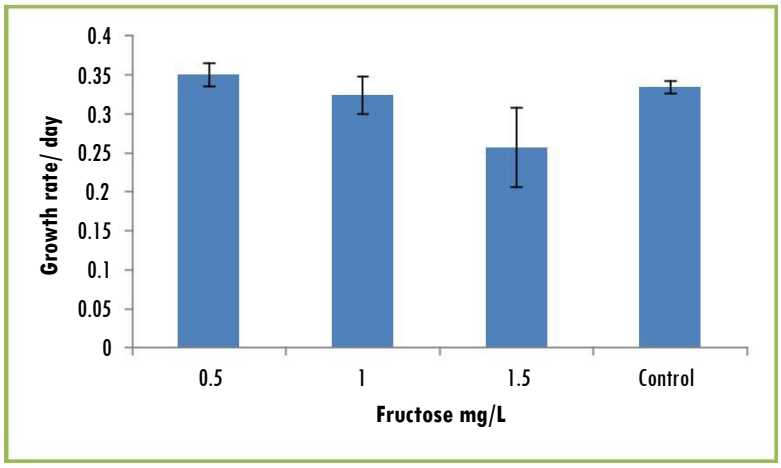

Fig.4 Effect of fructose on the growth rate of *N. salina*

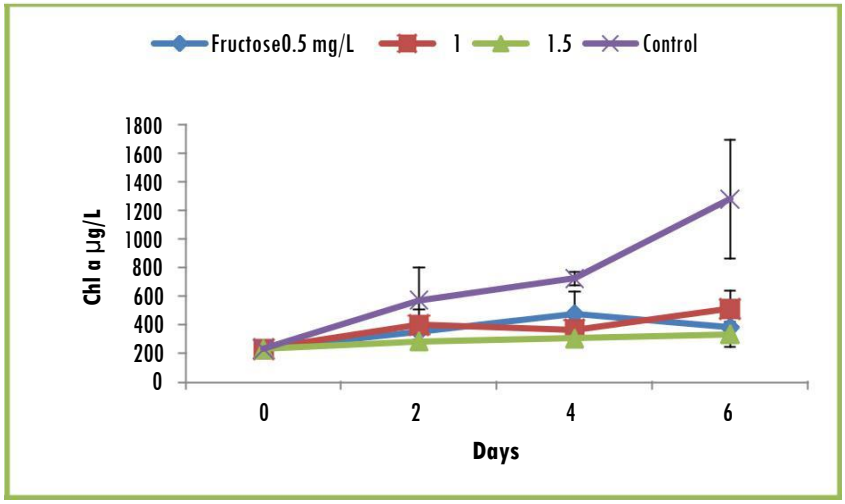

Fig.5 Effect of fructose on Chl *a* production in *N. salina*

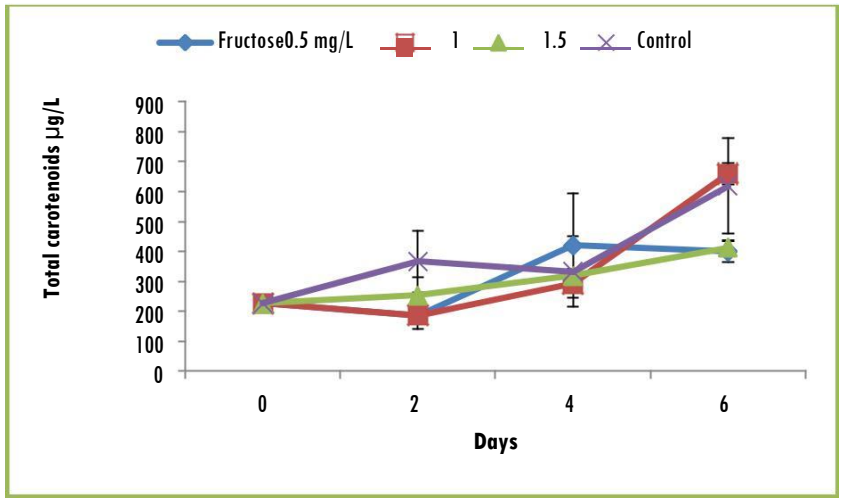

Fig.6 Effect of fructose on total carotenoid production in *N. salina*

Effect of lactose on growth rate of *N. salina* is presented in Fig.7. Maximum growth rate was noticed in cultures with 0.5 mg/L lactose (0.343/ day). Lactose free culture showed a growth rate of 0.334/day. ANOVA results showed that there is no significant difference between growth rates of *N. salina* in different lactose levels ($p = 0.786$, $p>0.05$). Increased production of Chl *a* was observed in cultures incorporated with 0.5 mg/L lactose (1679.7 µg/L) (Fig.8). Chl *a* production was 1250.6 and 1201 µg/L respectively in cultures incorporated with 1.0 and 1.5 mg/L lactose, while it was 1281 µg/L in the control. Fig.9 presents the effect of lactose on total carotenoid production in *N. salina*. While carotenoid production was higher in lactose incorporated cultures in comparison to the control, it reduced with increasing

concentrations. At 0.5 mg/L the production of carotenoid was 746.7 µg/L, whereas at 1.0 and 1.5 mg/L it was 686.7 and 553.3 µg/L respectively. In the control medium it was 620 mg/L.

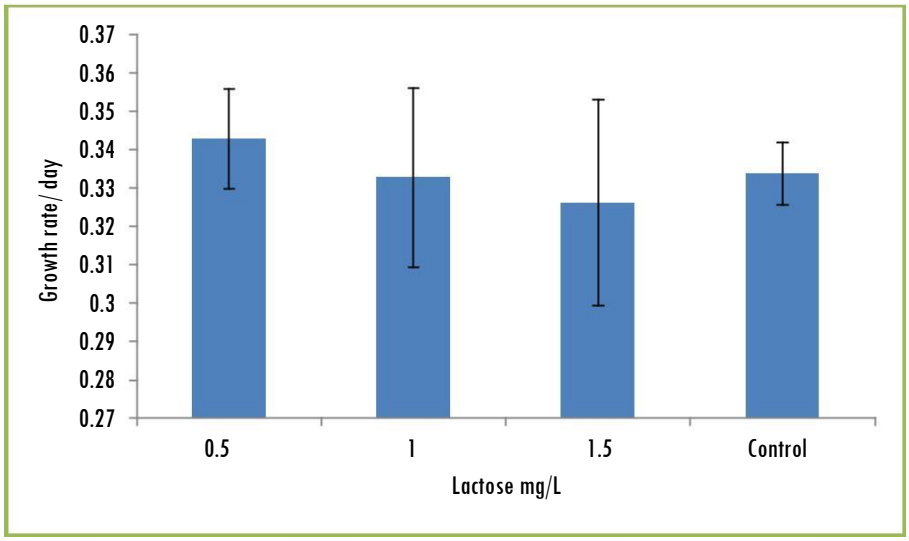

Fig.7 Effect of lactose on the growth rate of *N. salina*

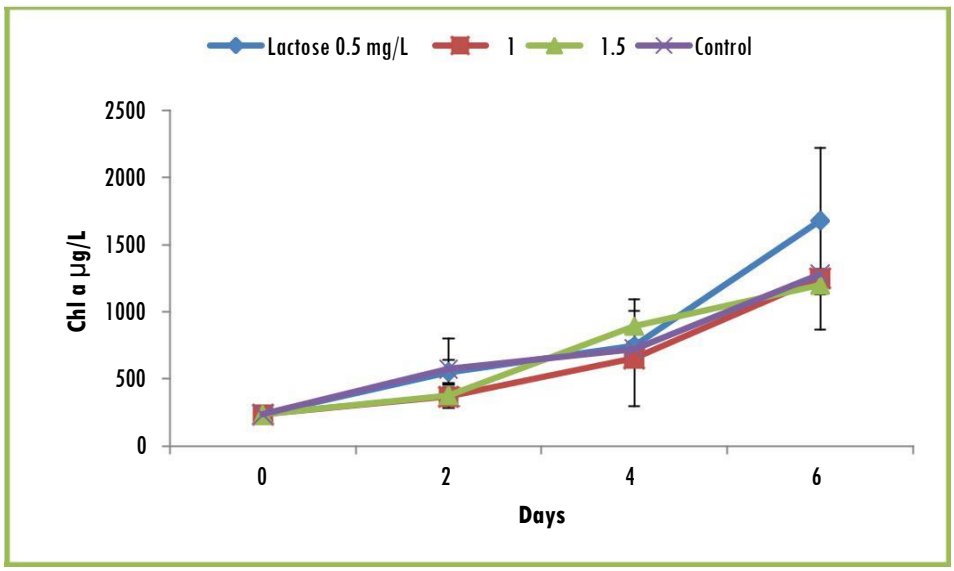

Fig.8 Effect of lactose on Chl *a* production in *N. salina*

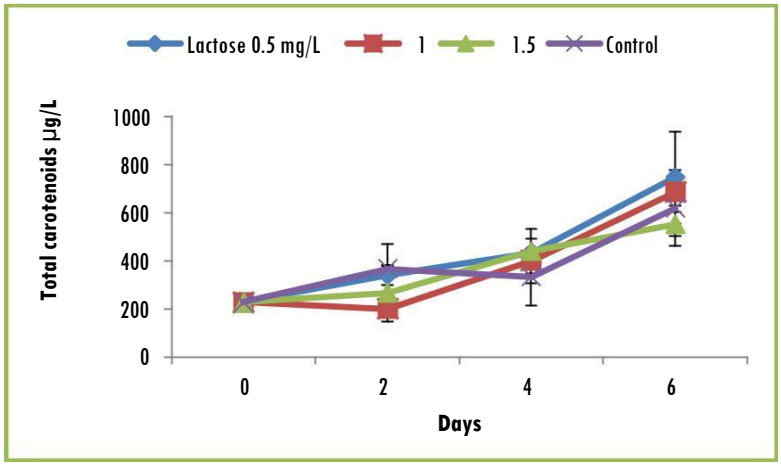

Fig.9 Effect of lactose on total carotenoid production in *N. salina*

Fig.10 presents the effect of sucrose on the growth rate of *N. salina*. While the growth rate at lowest concentration of sucrose tested (0.5 mg/L) was similar to that of the control (0.334/ day), increasing concentrations decreased the growth rate. ANOVA results showed that there is significant difference between growth rates of *N. salina* in different sucrose levels ($p = 0.000$, $p<0.05$). Addition of sucrose in the medium decreased the production of Chl *a* in *N. salina* cultures (Fig.11). Culture without sucrose produced 1281 µg/L Chl *a,* whereas cultures with sucrose produced very low quantities of Chl *a*. A decrease in carotenoid was noted in all the sucrose incorporated cultures with increasing concentration (Fig.12). Addition of 0.5 mg/L sucrose produced 453.3 µg/L carotenoid compared with 620 µg/L carotenoid in control.

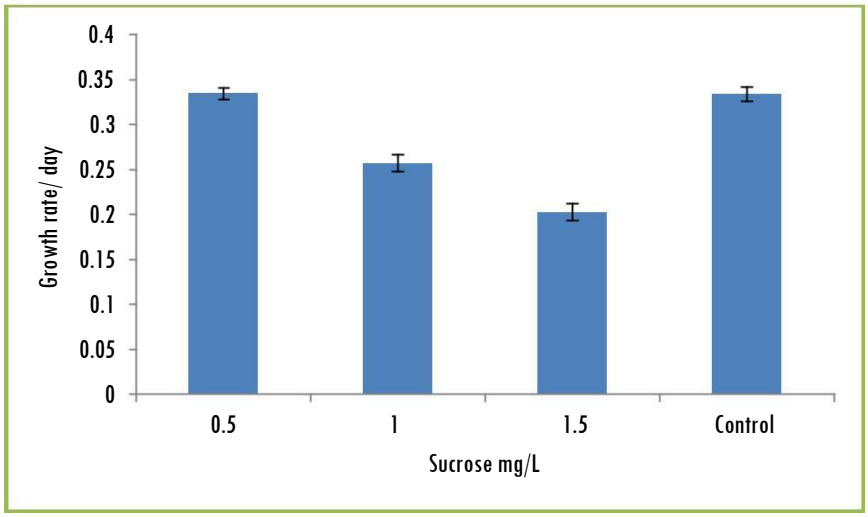

Fig.10 Effect of sucrose on the growth rate of *N. salina*

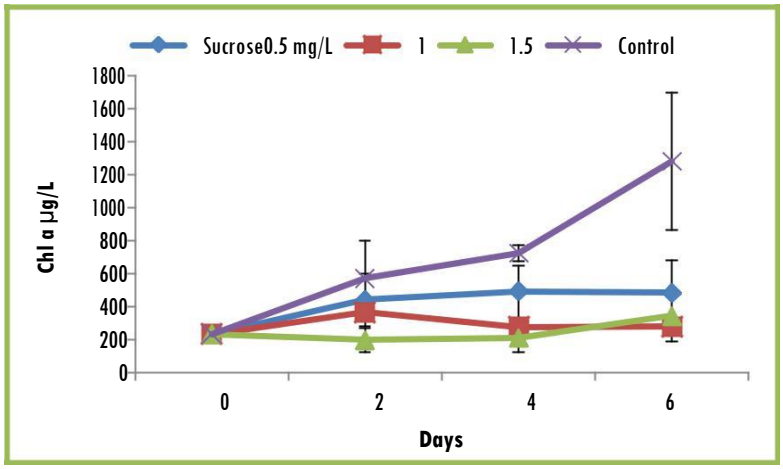

Fig.11 Effect of sucrose on Chl *a* production in *N. salina*

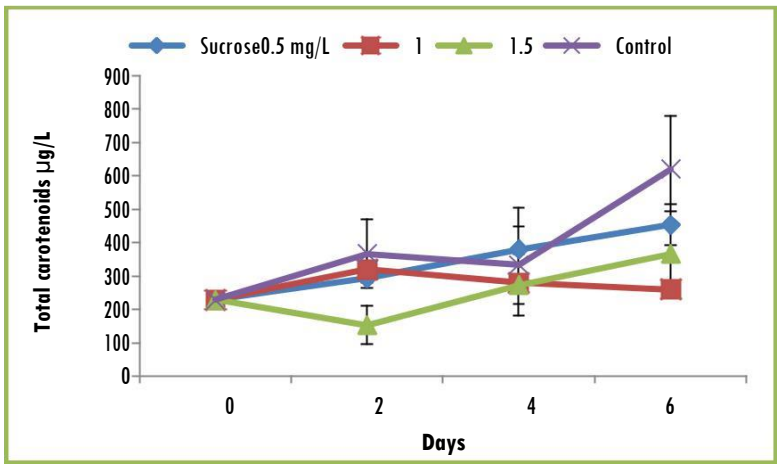

Fig.12 Effect of sucrose on total carotenoid production in *N. salina*

DISCUSSION

Carbon is essential for the growth of microalgae. Photoautotrophs fix atmospheric carbon dioxide and use this carbon for metabolism. Shi and Chen [27] observed that pigment content in photoautotrophic cultures were merely 1% of those in mixotrophic cells. However, in the present study, incorporation of organic carbon source into microalgal culture media only slightly affected the growth and pigment composition of *N. salina*.

Better growth rate was noted in cultures incorporated with 1 mg/L glucose. Shamala *et al.*[28] noticed a threefold increase in biomass of *Scenedesmus acutus* cultures in medium containing 0.05% (w/v) glucose. Shi *et al.* [29] found that biomass concentration of *Chlorella protothecoides* CS-41 increased from 4.9 to 31.2 g/L of

dry cells with increase in initial glucose concentration from 10-80 g/L. Velu *et al*. [23] showed that microalgae cultured in medium containing glucose showed the high culture density with fastest growth rate.

Production of Chl *a* decreased in cultures having glucose as a carbon source. This may be due to regulation of photosynthesis as there is availability of glucose in the medium. Chu *et al.*[30] also observed that *Ankistrodesmus convolutes* cultures grown on glucose had less chlorophyll and carotenoid contents than cultures grown on other carbon sources. Ogawa and Aiba [31] observed that effect of glucose in the culture medium depends on its concentration.

Carotenoid content was also lower in cultures containing glucose as carbon source. However, Ceron Garcia *et al.*[16] found that carotenoid content increases with increase in concentration of carbon source.

Fructose was also used as a carbon source in the present study. Good growth rate was observed in the medium with 0.5 mg/L fructose. Ceron Garcia *et al*. [16] noted good biomass increase in *Phaeodactylum tricornutum* when fructose was used as a carbon source. However, higher concentrations of fructose were not effective in biomass production. Hayward [32] showed that fructose did not stimulate the growth of *P .tricornutum*. Velu *et al.* [23]found low growth rate when fructose was used for mixotrophic culture of *Nannochloropsis salina, Dunaliella tertiolecta* and *Tetraselmis suecica*.

Pigment content was also influenced by the addition of fructose. In the present study carotenoid production was found to be high in 1 mg/L fructose added cultures. Such an increase in carotenoid content had already been reported[16]. But Chl *a* production decreased in cultures with fructose. Results of the present study do not agree with that of Ogawa and Aiba [31] who found that cellular contents of Chl *a* and *b* in *Scenedesmus acutus* deteriorated markedly in mixotrophy.

0.5-1.5 mg/L lactose was used in the study as carbon source. Growth rate decreased with increase in lactose content. 0.5 mg/L lactose produced good growth. Velu *et al*. [23] noted a positive effect of lactose on the growth rate of *N. salina* and *T. suecica*, while Ceron Garcia *et al*. [16] had found no significant effect of lactose in cultures of *Phaeodactylum* sp. Chl *a* production was high in lactose incorporated cultures.Carotenoid production was also positively influenced by the presence of lactose in the medium.

In the present study increasing concentration of sucrose was accompanied by decreasing growth rate of *N. salina*. Pigment production was also found to have decreased in presence of sucrose. In contrast to these finding, Velu *et al.*[23] observed an increase in biomass yield in *N. salina, Dunaliella tertiolecta* and *Tetraselmis suecica*.

CONCLUSION

Incorporation of organic carbon sources into microalgal culture media affected the growth and pigment composition of *N. salina.* Better growth rate was noted in cultures incorporated with low concentrations of organic carbon sources. Pigment production was also negatively influenced by increasing concentrations of carbon source. *N. salina* could grow better in mixotrophic cultures with low concentrations of organic carbon sources.

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