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Influence of different concentrations of sodium bicarbonate on growth rate and chlorophyll content of *Chlorella salina*

*Reeta Jayasankar and K. K.Valsala

Central Marine Fisheries Research Institute, Cochin-682 018, India.
**E-mail: reetajayasankar@yahoo.com*

Abstract

Chlorella salina was grown in enriched medium supplemented with different concentrations of sodium bicarbonate to study the influence of inorganic carbon (C_i) on its growth and chlorophyll content. Linear growth was observed on the treatment with 0.004 and 0.005 M sodium bicarbonate whereas exponential growth was observed in control and 0.001 to 0.003 M bicarbonate treatment. The growth rate was maximum in 0.005 M concentration with a 1/ln value of 0 .270 $\pm$ 0.01 on 15 days of culture. Increase in growth was observed with the increase of concentration of sodium bicarbonate from 0.001 to 0.005 M. Growth rate in 0.005 M concentration was 70% more than that of the control. There was steady decline of photosynthetic pigments (chl *a* & chl *b*) in both control and treatment in the initial period of growth. The decline was up to 4th day in the treatments from 0.002 to 0.005 M concentration whereas it was up to 7th day in control and 0.001 M treatment. Chl *b* was found to be steady from 7th to 15th days of treatment. Maximum chl *a* was observed in 0.005 M treatment (7.9 μg/ml) on 15th day and minimum during 4th day of culture (0.6 μg/ml). Chl *a* to chl *b* ratio showed increase in all the treatments until 13 days of culture period. In control and lower concentration of C_i treatment, the ratio increased to more than 2 after 9th day, whereas similar observation was found on 4th day of culture in 0.004M and 0.005 M treatments. This result leads to the conclusion that there is a need to augment inorganic carbon along with the enriched medium during the initial period of culture of *Chlorella salina* to improve the specific growth rate.

Key words: *Chlorella salina,* chlorophyll content, sodium bicarbonate, growth rate, inorganic carbon

Introduction

Inorganic carbon (C_i) dissolved in seawater is mostly composed of high concentration of bicarbonate ion and low concentration of carbon dioxide (Israel and Gonzalez, 1996). The microalgae utilize bicarbonate as the external source of carbon for photosynthesis (Dixon *et al.*, 1987; Munoz and Morrett, 1989; Beer, 1994). Few algae are capable of uptake of carbon dioxide directly (Badger, 1985; Raven, 1991) while others convert bicarbonate to carbon dioxide either inside the plasmalemma (Dixon *et al.*, 1987) or externally allowing only bicarbonate to diffuse into the cell (Badges *et al.,* 1980). Photosynthesis in microalgae in a carbon limiting environment displays characteristics like C_4 type plants with much higher affinity to CO_2 but unlike CO_2 enrichment in C_4 plant, the microalgae operate by accumulating inorganic carbon intracellularly and the uptake is driven by energy coupled C_i transport system (Yingjun and Martin 2006). Enzyme carbonic anhydrase is associated with the process of reversible hydration of carbon dioxide helping to increase the efficiency of photosynthesis in microalgae (Suzuki *et al*., 1994).

Chlorella salina is a single celled green alga with rich source of energy as the photosynthetic efficiency theoretically reaches 8% which is comparable with the higher crops like sugarcane. It is also an attractive food source of many marine larvae due to its nutritional package of unsaturated fatty acids, carbohydrate, minerals and other essential nutrient content and has great demand in aquaculture industry as live feed.

Material and methods

Approximately 100 ml of *Chlorella* was concentrated by centrifugation and then diluted to 4 ml with sterilized seawater. Five hundred microlitre of the samples were added to each flask containing 300 ml of seawater enriched with Walne's medium. Sodium bicarbonate ranging from 0.001 to 0.005 M concentration was added to different flasks. Duplicate was maintained in each treatment. Enriched medium without sodium bicarbonate was considered as control. After inoculation, the flasks were kept under controlled environmental conditions having light intensity of 1000 lux, temperature of 24-25^{0}C and photoperiod of 16:8 h light and dark cycle. The growth rate was expressed in percentage of transmittance by measuring the turbidity at three different wavelength of light in blue, green and red region at 430, 540 and 678 nm respectively. The growth curve was drawn by taking the average value of all the transmittance and converting them to negative logarithmic value. Photosynthetic pigment was extracted after centrifuging 5 ml of the *Chlorella* from the enriched medium and kept overnight in 90% acetone. The solution was vortexed and again centrifuged at 10,000 rpm in Hitachi refrigerated centrifuge at 4^{0}C. Optical density of the supernatant was measured at 630, 645 and 663 nm. Estimations of Chl *a* and Chl *b* were carried out by following standard procedure (Jeffrey and Humphrey, 1975). Two replications were taken for each treatment on 2nd, 4th, 7th, 9th, 13th and 15th days of culture. Data were analyzed by Analysis of Variance using SYSTAT 7.0 and the results were interpreted statistically.

Results

There was a steady increase in the growth of *Chlorella* from 2nd to 15th day of culture in both control and treatment as observed in the turbidity study expressed in the negative logarithmic of the rate of transmittance (Fig.1). The growth was found to be the lowest in the control and was higher with high carbon concentration. There was no marked difference in the growth in all the treatments up to 4 days of inoculation. From the 7th day onwards, the growth showed linear progress in the treatment

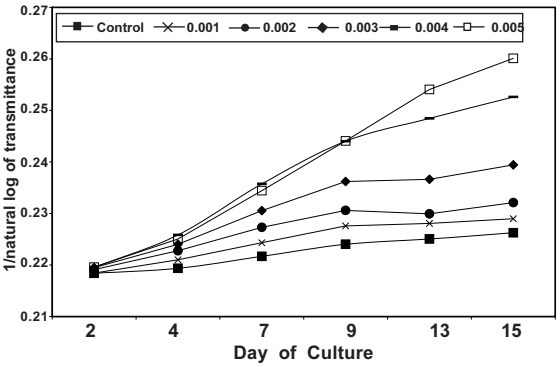

Fig. 1. Growth rate of *Chlorella salina* treated with different concentrations of sodium bicarbonate

supplemented with high concentration of C_i. At 0.005 M enrichment a linear progress of growth was observed rather than a sigmoid curve. In control and treatments from 0.001 to 0.003 M concentration, the steady state growth occurred on 9th day of culture whereas under the treatment of 0.004 and 0.005 M the growth increased until 15th day of culture. There was significant variation in growth ($p<0.01$) between treatment and the control with a maximum negative logarithmic value of transmittance of 0.270 at 0.005 M and minimum of 0.218 in controlled condition (Fig. 1).

It was observed that both chl *a* and chl *b* declined immediately after the inoculation and persisted until 7th day of culture period in the control and in 0.001 M sodium bicarbonate whereas in other treatments the decline was only until 4th day of inoculation. Subsequently it increased. Sharp increase of chl *a* was found in the treatment with 0.005 M followed by 0.004 M sodium bicarbonate till 15 days of culture period whereas a steady state was observed in other treatments and also in the control after 9th day of culture. Significant variation was observed among the treatments and in control with a maximum chl *a* content in treatment with 0.005 M concentration on 15th day of culture (7.9 μg/ml) and minimum in control on 4th day of culture (Fig. 2). On 2nd day after inoculation, the maximum chlorophyll content was found in the control followed by treatment with lower concentration (0.001 M), which reduced in higher concentration of bicarbonate and was

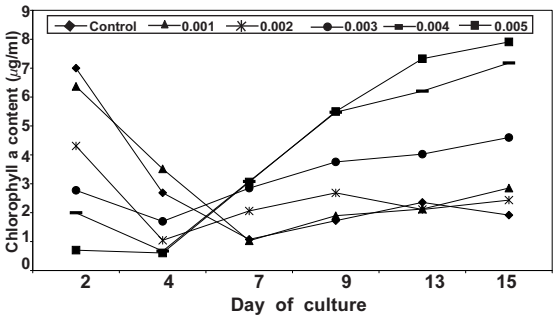

Fig. 2. Chlorophyll *a* content of *Chlorella salina* treated with different concentrations of sodium bicarbonate

lowest in 0.005 M concentration. Thus the lowest chl *a* content was observed in the 0.005 M treatment on 4th day after inoculation. But in the control the decline persisted for 7 days. There was not much significant variation in chl *a* content among the treatments but the variation was significant in different days of culture period ($p<0.01$).

Chl *b* also showed a similar trend with an initial decline until 4th and 7th day but there was not much difference in the subsequent days. Among the treatments the chl *b* was higher in higher concentration (0.005 M). Significant variation was observed in chl *b* among the treatments and control (Fig. 3) and between different days of culture period ($p<0.01$).

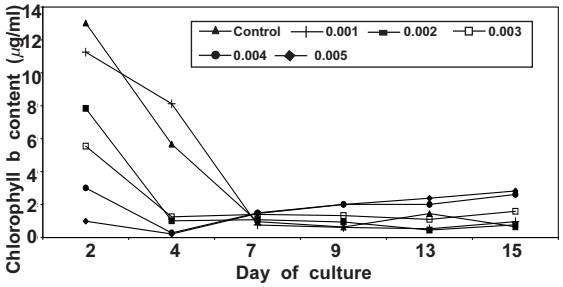

Fig. 3. Chlorophyll *b* content of *Chlorella salina* treated with different concentrations of sodium bicarbonate

The Chl a/b ratio sharply increased in the control and low concentration of sodium bicarbonate (0.001 M) from 7th to 13th day of inoculation, but declined subsequently. In the treatment with 0.004 and 0.005 M concentration, the ratio sharply increased on 4th day followed by a decline on 7th day. From 9th to 13th day it increased marginally and then declined on 15th day. It was observed that the ratio of chl *a*/chl *b* showed a declining trend in all the treatments on 15th day (Fig. 4). In all the other treatments excluding the control, the ratio increased until 15th day of culture with a maximum value on 13th day. Significant difference was observed between the treatments and between the days of culture ($p<0.01$).

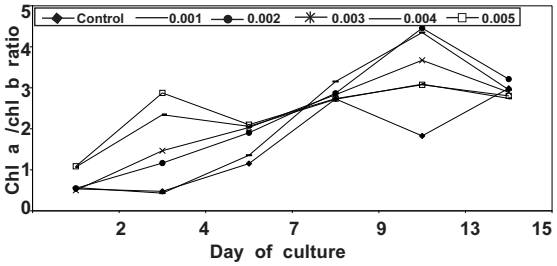

Fig. 4. Chl *a*/chl *b* ratio of *Chlorella salina* subjected to different treatment of sodium bicarbonate

While comparing the growth with control, it was observed that there was 5 to 70% increase in growth in all the treatments with the highest value in 0.005 M concentration of sodium bicarbonate in 15 days of culture period.

Discussion

Growth rate of microalgal population is a measure of increase in biomass over time and is determined from the exponential phase. The duration of the exponential phase depends upon the size of the inoculums, their growth rate, culture condition and the enriched medium where it grows. The growth of microalgae not only depends on the temperature, light and nutrient availability, but also has a direct impact on the available carbon in the culture medium. The carbon to chlorophyll ratio is a sensitive indicator of the physiological state of microalgae (Geider, 1987). In the present experiment on growth, the pigment constituents were taken as the indicator of the physiology of microalgae influenced by inorganic carbon. It is reported that at low cell density, the addition of inorganic carbon will reduce the pH thereby reducing the growth in the initial phase of inoculation (Geider, 1987). Similar observation was made in the present experiment as the cell density

was very low (88 x 10^4 cells) when diluted to 300 ml of culture medium with a concentration of less than 1500 cells/ml. This could have influenced a change in the pH and lowered the pigment concentration in higher C_i enrichment in the initial period.

Pesheva *et al.* (1994) observed that the growth of the marine microalga, *Chlorococcum littorale* suppressed for the first 3-4 days after inoculation with medium supplemented with 40% carbon dioxide. However logarithmic growth was observed after that period. According to them, the growth rate was influenced by the suppression of PSII activity whereas the PSI activity increased (Iwasaki and Miyachi, 1996). Increase of PSI/PSII ratio will support the energy supply for ATP synthesis through cyclic photophosphorylation. Pronina *et al*. (1993) also observed photosynthesis by increasing the concentration of carbon dioxide with intracellular acidification. Similar observation was made in the present experiment. Increase in biomass was due to linear growth than exponential growth with respect to time and is directly proportional to the input of carbon dioxide as observed in the treatment with 0.004 and 0.005 M carbon dioxide.

Carbon to chlorophyll ratio is also a sensitive indicator of the physiological state of microalgae (Geider, 1987). With constant supply of light and temperature, there is a linear relationship between carbon input and chlorophyll content. Similar result was obtained in the present experiment where the chl a content showed a linear increase at 0.005 and 0.004 M concentration of sodium bicarbonate.

Photosynthetic pigments in green algae constitute mostly chl *a* and chl *b*. The physiological condition of microalgae can immediately influence the pigment constituents. The ratio between chlorophyll *a* and chlorophyll *b* indicate the physiological status of the microalgae. In the present experiment, an immediate decline of chl *a* and chl *b* in all the treatments including control was found, which may explain the change in pH while acclimatizing the plant to new enriched medium .The decline was more in control and 0.001M treatment of sodium bicarbonate and persisted until 7th day of culture. The decline was less as the concentration of sodium bicarbonate increased. According to Shiraiwa *et al.* (1993), there are several microalgae, which have CO_2 concentration systems that maintain pH balance intracellularly and generally increase pH extracellularly. *Chlorella salina* showed direct interaction with inorganic carbon by increasing the growth rate and the reduction in chlorophyll pigments from 0.002 M to 0.005 M sodium bicarbonate treatment. This might be due to the neutralization of the plant to overcome the change in pH extracellularly for at least 4 days to overcome the intracellular changes of pH.

Israel and Gonzalez (1996) reported that enriched medium led to decline in pH with the formation of carbonic acid due to extracellular carbonic anhydrase. Ion of sodium bicarbonate may not be able to neutralize it. Similar observations were made in the present experiment. The ratio of chl *a*/chl *b* showed a very low value for the control and the treatment with 0.001 M sodium bicarbonate corresponding to high concentration of chl *a* and chl *b* . This increase of chlorophyll did not influence the growth considerably. Further there was significant difference in growth in different treatments with control. Perhaps there was carbon limitation while culturing *Chlorella salina* in control and with low carbon input. Perceptible changes in growth were observed in all the treatments after 4 days of inoculation. Thus input of carbon intermittently along with enrichment of seawater will enhance the growth to a greater extent in situations of carbon limitation.

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