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Sodium bicarbonate augmentation enhances lutein biosynthesis in green microalgae *Chlorella pyrenoidosa*

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ABSTRACT

The present study was carried out to investigate the effects of different concentrations of sodium bicarbonate (0, 50, 100, 150 and 200 mM) on the biosynthesis of lutein and lipids and also the expression of genes involved in lutein carotenogenesis in *Chlorella pyrenoidosa*. The algal extract from cultures treated with different concentrations of sodium bicarbonate was analyzed using high-performance liquid chromatography (HPLC) for lutein quantification. HPLC results revealed a three-fold increase of lutein in *C. pyrenoidosa* culture grown in 100 mM concentration (4.84 ± 0.56 mg/g of DCW), followed by 50 mM concentration (4.21 ± 0.11 mg/g of DCW) supplemented cultures compared to 150 mM and 200 mM concentration (2.32 ± 0.093 mg/g of DCW and 2.31 ± 0.114 mg/g of DCW) and control culture (1.45 ± 0.18 mg/g of DCW). The expression analysis of phytoene desaturase, lycopene cyclase and carotenoid hydroxylase genes also showed a significant up regulation in their expression when supplemented with sodium bicarbonate. Lipid yield was observed to be increased significantly when supplemented with sodium bicarbonate. Therefore, sodium bicarbonate (100–150 mM) can be used as a potential dissolved inorganic carbon source for enhancing lutein and lipid production during large scale cultivation of *C. pyrenoidosa* cultures.

1. Introduction

Microalgae have been recognized as a potential source of livestock feed, lipids, carotenoids, pharmaceutical and nutraceutical compounds, alternative fuels, cosmetics, foods and other high value secondary metabolites (Spolaore et al., 2006; Weikel et al., 2012). One of the most efficient ways of improving microalgal industry is by increasing the production of high value products at low cost. This can be achieved by exploiting the fact that microalgae tend to produce high yield of lipids and carotenoids as defense mechanisms to protect themselves from oxidative damage when exposed to stress (Couso et al., 2012; Ip and Chen, 2005; P. Sharma et al., 2012). Modifications to the nutrients in the culture media such as supplementation, limitation and depletion play a major role in the enhancement of biochemical composition of both marine and fresh water microalgae (Sampathkumar et al., 2019; Srinivasan et al., 2018, 2015). This is possible due to altering in the metabolic pathways by the microalgae, which results in the production of lipids, proteins and carbohydrates. Certain algal species such as *Chlorella* sp. (Illman et al., 2000; Liu et al., 2008), *Dunaliella* sp. (Chen and Jiang, 2009; Giordano et al., 2002) and *Nannochloris* sp. (Takagi et al., 2000) have been reported to possess the ability to amass high quantity of lipids and carotenoids under optimum conditions (Heydarizadeh et al., 2013; K. K. Sharma et al., 2012). Carotenoids are colored secondary pigments with 40 carbon chain structures that are found to give wide range of colors in fruits and vegetables. Carotenoids mainly function by protecting the photosynthetic apparatus, as scavengers of Reactive Oxygen Species (ROS) that are produced during photosynthesis and also stabilizing the folds of proteins involved in photosynthesis. They also protect proteins, chlorophylls, nucleic acids and lipids from the ROS produced during absorption of sunlight in the light harvesting complexes by functioning as antioxidants (Ahmed et al., 2015; Ibañez and Cifuentes, 2012). Due to their antioxidant properties, they have been reported to play vital role in human health as protectors against cardiovascular problem, rheumatoid arthritis, cancers and amyotrophic lateral sclerosis (Fitzgerald et al., 2013; Ibañez and Cifuentes, 2012). A study by Maeda et al. reported that carotenoids possess anti-diabetic, anti-obesity and anti-inflammatory properties (Maeda et al., 2005). There are more than 600 naturally available carotenoids from different sources (plants,

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microalgae, bacteria, fungi, etc.). Lutein is one such carotenoid that for which studies has shown to possess high antioxidant properties and whose presence reduces the danger of early onset of cataract and age related macular degeneration in human eye (Robson et al., 2006). Lutein has also been reported to have many applications related to human health such as anticancer (Demmig-adams and Adams, 2002), anti-biofilm (Sampathkumar et al., 2019), treatment for ameliorating cardiovascular diseases (Weikel et al., 2012), etc. Lipid is another economically valuable product produced by *Chlorella pyrenoidosa* apart from lutein. When subjected to stress, microalgae have the ability to assimilate high concentration of lipids (Ramesh Kumar et al., 2019). Many studies have been carried out extensively to enhance the production of lipids and fatty acids in microalgal cultures. A study that was carried out by Kamyab et al., (2016) on the effect of glucose (carbon source) and photons (light intensity) on the lipid biosynthesis and biomass in *Chlorella pyrenoidosa* that are abundantly available in the Malaysian palm oil sewage (Kamyab et al., 2016). Another study by Sharma et al. (2019) screened for nearly 20 microalgal consortiums, where the microalgae cultures were obtained from different areas of Rajasthan and Haryana. They had observed an increase in lipids biosynthesis in the consortiums and therefore optimized these consortiums for biodiesel application (Sharma et al., 2019). Composition of growth medium plays a major parameter influencing microalgal growth. Allen Medium, BG11, F-2 Medium, Bold Basal Medium, MA Medium, Fogg's Medium and Noro Medium, etc. are the growth mediums that are most used for cultivation of microalgae (Kumar et al., 2019; Muñoz and Guieysse, 2006; Sharma et al., 2018; Wang et al., 2014). The average yield of lipids in algal cells is between 1 to 70%, which also functions as a novel precursor for biodiesel production (Kamyab et al., 2019). Whereas, microalgae grown in artificial media showed an yield of more than 80% lipid content (Chisti, 2007; Kamyab et al., 2019). The total lipid content in most microalgae ranges within 1–85% of their dry weight. Some may even achieve higher than 40% under nutrient-limited conditions. Apart from microalgal cultures, some species of cyanobacteria have also been studied for their ability to produce value added products that can be used for biofuel production (Garlapati et al., 2019). *Chlorella pyrenoidosa* has been studied extensively for its high lipid accumulation under different stress conditions and reported as a potential and efficient source of lipids that can be used for energy production (Chi et al., 2019; Kamyab et al., 2017; Nigam et al., 2011; Rai et al., 2015). Microalgae use photosynthesis to utilize CO_2 efficiently, almost 10–50 times more than that of the plants (Anto et al., 2019). Inorganic carbon sources helps in enhancing the total carotenoid and lipid content by improving the growth rate, which makes it one of the main components in the growth media of microalgae (Gardner et al., 2013; Juneja et al., 2013). Two forms of carbon dioxide, namely gaseous carbon dioxide and inorganic carbon (sodium bicarbonate), can be assimilated by and used by. Few algal species can uptake carbon dioxide in its gaseous form and utilize it as a carbon source for growth and other species convert gaseous carbon dioxide into its bicarbonate form by a chemical disequilibrium and utilize it in photosynthesis. Moreover, the elevated pH due to addition of carbon helps in reducing the contamination by other microorganisms and to functions as buffer to maintain growth of the algal species (Richmond et al., 1982; Sathasivam and Juntawong, 2013). When compared to gaseous carbon dioxide, inorganic carbon or sodium carbonate is more economically feasible and easy to transport. It is also considered to be a better supplement for growth of microalgae than that of gaseous carbon source. Since sodium bicarbonate ($NaHCO_3$) has a very high solubility (96 g/l H_2O at 20 °C), it generates a high concentration of bicarbonate ions (HCO_3^-) when it is dissolved in the culture media, with a slight increase in the pH. The buffer capacity of sodium ions helps in supersaturating of dissolved inorganic carbon and keeps a constant dissolved CO_2 concentration under atmospheric pressure. From the different inorganic carbon sources, most microalgae can use only either aqueous CO_2 or HCO_3^- for their growth and these two carbon sources will be, from here on, denoted as dissolved inorganic carbon or DIC. Therefore, in this study, $NaHCO_3$ was used as an inorganic carbon source for autotrophic growth. A primary objective of this study is to investigate the autotrophic growth of *Chlorella pyrenoidosa* under different concentrations of dissolved inorganic carbon controlled with sodium bicarbonate at constant shaking of the culture media. *C. pyrenoidosa* have been traditionally used as a source for enhanced production of lipids and fatty acids under different stress conditions but there are not many studies that has explored *C. pyrenoidosa* as a potential source of lutein and studied the production of lutein under different stress conditions, especially under sodium bicarbonate supplementation. Thus, the current investigation aims to enhance the biomass production and biosynthesis of intracellular pigments of the fresh water microalgae, *C. pyrenoidosa*, by supplementation of sodium bicarbonate.

2. Materials and methods

2.1. Culture strain and growth

The algal strain used in this study, *Chlorella pyrenoidosa* (NCIM-2738) was obtained from National Collection of Industrial Microorganisms, Pune, Maharashtra, India. The culture was then cultured in Fog's medium (as per NCIM guidelines), containing 0.2 g of $MgSO_4{\cdot}7H_2O$, 5 mL of Fe-EDTA solution (745.0 mg of Na_2EDTA and 557.0 mg of $FeSO_4{\cdot}7H_2O$ for 100 mL), 0.2 g of K_2HPO_4, 0.1 g of $CaCl_2{\cdot}H_20$, micronutrient solution (286.0 mg of H_3BO_3, 181.0 mg of $MnCl_2{\cdot}4H_2O$, 22.0 mg of $ZnSO_4{\cdot}7H_2O$, 39.0 mg of $Na_2MoO_4{\cdot}2H_2O$, 8.0 mg of $CuSO_4{\cdot}5H_2O$ for 100) (Kanaga et al., 2016). The culture was grown in orbital shaking incubator at 150 rpm and 27 °C with 16:8 light/dark cycles and an illumination of 42 μmol m^{-2} s^{-1}.

2.2. Experimental conditions

The effect of addition of varying concentrations of sodium bicarbonate (50 mM–200 mM) on the production of lipids and carotenoids, were carried out using fog's medium. Quantification of chlorophyll pigments and total carotenoids were carried out at regular intervals (5 days) and on day 30, cultures were harvested and used for quantifying lutein using HPLC.

2.3. Effect of sodium bicarbonate supplementation on growth, biomass and pH

The growth study was carried out with 20 mL of control and stress induced and its absorbance was quantified at 750 nm using Shimadzu UV–Visible spectrophotometer. The growth study was carried out at an interval of 5 days for 30 days. The dry biomass was calculated by centrifuging 20 mL of culture at 12,000 rpm for 10 min. The pellet was then dried at 100 °C, overnight and weighed. The pH of the medium was determined at regular intervals using EcoTest pH meter (Thermo Scientific).

2.4. Effect of sodium bicarbonate supplementation on chlorophyll pigments and total carotenoid production

The quantification of total carotenoids, chlorophyll *a* and chlorophyll *b* was done with 20 mL of treated and untreated cultures. The cells were centrifuged at 15000 rpm for 5 min. The pellet was washed twice with distilled water and the pigment extraction was carried out by adding 1 mL of 80% acetone to the pellet. The pellet was sonicated and then incubated in dark for 4 h and centrifuged again. The extract was then analyzed for the different pigments (Chlorophyll *a*, Chlorophyll *b*, and Total carotenoids) at 665 nm, 642 nm and 470 nm respectively, using Shimadzu UV–Vis spectrophotometer (Lichtenthaler and Wellburn, 1983). The pigments were then quantified using the formulas given below,

$$Chlorophyll\ a\ (\mu g/mL) = 11.75\ A_{665} - 2.35\ A_{642} \text{ -} \quad (1)$$

$$Chlorophyll\ b\ (\mu g/mL) = 18.61\ A_{642} - 3.96\ A_{665} \text{ -} \quad (2)$$

$$Total\ carotenoids\ (\mu g/mL) = (1000\ A_{470} - 2.270\ \text{Chl}\ a - 81.4\ \text{Chl b})/198 \text{ -} \quad (3)$$

2.5. Lutein extraction and quantification using HPLC

For extraction of lutein, 2 mg of lyophilized control and stress induced cells were used. Lutein extraction was carried in two phases, namely, saponification and extraction. In the saponification step, the *C. pyrenoidosa* cells were treated with potassium hydroxide (KOH), which was found to enhance the efficiency of lutein extraction (Saguy et al., 2006). The microalgal biomass was mixed with 40% (w/v) potassium hydroxide solution and the mixture was subjected to cell lysis and incubated at 40 °C for 40 min. The mixture was further incubated at 4 °C in dark for 6 h. After that, second phase of extraction was carried out using organic solvents. Extractions were carried out repeatedly to recover maximum quantity of lutein from the microalgae. The alkaline treated cells were mixed with the organic solvents in the ratio of 2:1 (v/v). This mixture was then centrifuged at 5000 rpm for 5 min. The supernatant (organic phase) was recovered and subjected to multiple extractions using acetone. The acetone extract was then measured for its lutein content using a reverse phase silica C18 column Shimadzu LC 20A HPLC system with a dual pump system (LC-20AT) and UV–Visible detector (SPD-20A), using an isocratic solvent as mobile phase consisting of acetonitrile-methanol-dichloromethane in the ratio 70:10:20, at a flow rate of 1.0 mL and detected at 467 nm (Srinivasan et al., 2018).

2.6. Extraction and quantification analysis of lipids

A solvent mixture containing chloroform and methanol in the ration 1:1 were added to the freeze dried *C. pyrenoidosa* cells from different treatments (Ryckebosch et al., 2012). The cells were then sonicated for 15 min and the solvent phase was collected and dried at 40 °C. The total lipid content was determined gravimetrically.

2.7. Total protein extraction and quantification

The *C. pyrenoidosa* culture was centrifuged and the pellet was resuspended in pre-chilled phosphate buffer (pH 7.0). Cell lysis was carried out using ultrasonication and the homogenate was centrifuged for 15 min at 10,000 rpm. The supernatant was collected and its total protein content was quantified by Bradford method, using bovine serum albumin as a standard (Bradford, 1976).

2.8. Effect of sodium bicarbonate supplementation on intracellular antioxidant enzyme activity

The crude protein extract from algal cultures were used for investigating the antioxidant enzyme activity. Algal cells were harvested and crushed to powder using liquid nitrogen. Grounded samples (0.5 g) were homogenized in 10 mL of solution containing 1% (w/v) polyvinylpyrrolidone and 50 mM of potassium phosphate buffer (pH 7.8) and was incubated for 10 min at 4 °C. The homogenate was filtered and centrifuged at 6000 rpm for 15 min at 4 °C. The supernatant containing the crude protein was stored at 4 °C for further analysis. Superoxide dismutase (SOD) activity assay was carried out by observing the reduction in absorbance caused by inhibition of nitro-blue tetrazolium (NBT) photochemical reduction reaction as described by Beauchamp and Fridovich (Beauchamp and Fridovich (1971).

2.9. Effect of sodium bicarbonate supplementation on expression of genes involved in lutein carotenogenesis

Gene expression studies for pds (phytoene desaturase), lcy (lycopene cyclase) and chy (carotenoid hydroxylase) were done using RT-PCR, where, β-actin was used as normalization control. RNA isolation was carried out using TRIzol method. Quantification and purity of RNA samples were measured using nanodrop method from ThermoFisher, USA. cDNA synthesis was performed with High capacity Reverse Transcription kit by Thermo Fisher Scientific. The primer sequences from 5′ to 3′ are given in Table S1 (supplementary file).

2.10. Statistical analysis

All experiments were carried out in triplicate. Two-way ANOVA was performed as statistical analysis, followed by Bonferroni post-test analysis using Graph pad Prism v5.0. The statistical significance of the results was validated if the p value was <0.05.

3. Results and discussions

3.1. Impact of Sodium bicarbonate supplementation on biomass, growth and pH

The effect of varying concentrations of sodium bicarbonate (0 mM–200 mM) was observed on the growth of *C. pyrenoidosa* by measuring the absorbance at 750 nm, along with the pH of the medium. The growth and biomass was found to be enhanced in the group treated with 100 mM of sodium bicarbonate, followed by enhancement in 150 mM concentration (Figs. 1 and 2). The pH of the media for the control samples was initially optimum at 7.5 and gradually increased with the days. On the other hand, the media pH of the group treated with different concentrations of sodium bicarbonate was observed to be almost constant at 9.7 (Fig. 3). For photoautotrophic microalgae such as *C. pyrenoidosa*, supply of ample quantity of inorganic carbon is imperative to carry out normal growth and photosynthesis. This can be accomplished by supplementation of carbon either as bicarbonate or gaseous CO_2 to the microalgal growth media. Bicarbonate form of carbon supplementation is highly advantageous in commercial applications where there is a limitation in supply of adequate CO_2. Bicarbonate also has higher solubility than gaseous CO_2 and thus reduces the problem associated to low retention time of carbon in the growth media (Hsueh et al., 2007). However, the biochemical composition and the metabolic efficiency might vary based on the difference in the utilization of bicarbonate by different microalgal species (Giordano et al., 2005). The pH of the experimental growth medium with bicarbonate indicated a pH of 9.4. This observation can lead to the understanding that HCO^{3-} is utilized as a carbon source to avoid the limitations of using carbon via carbon concentrating mechanisms (Badger, 2003). Similar results have been observed in several other microalgal species where a high pH condition has caused lipid accumulation (White et al., 2013). Peng and his colleagues proved that combination of 160 mM NaHCO3 and pH 9.5 showed maximum microalgal growth when compared with 160 mM/pH 8.5 and 160 mM/pH 7.5 (Peng et al., 2015).

3.2. Impact of Sodium bicarbonate supplementation on chlorophyll a, chlorophyll b and total carotenoids

Similar to pH and growth, the chlorophyll content of the cultures was measured at 5-day intervals by UV–visible spectrophotometry. The chlorophyll *a* (chl a) content was quantified at the wavelength 662 nm and chlorophyll *b* (chl b) content was measured at 645 nm. The observations were plotted using GraphPad Prism (v 5.0). In both plots, the maximum chlorophyll *a* (4.038 ± 0.60 μg/mL) and chlorophyll *b* (3.18 ± 0.20 μg/mL) yields were observed in the cultures treated with 100 mM concentration of sodium bicarbonate in comparison to the

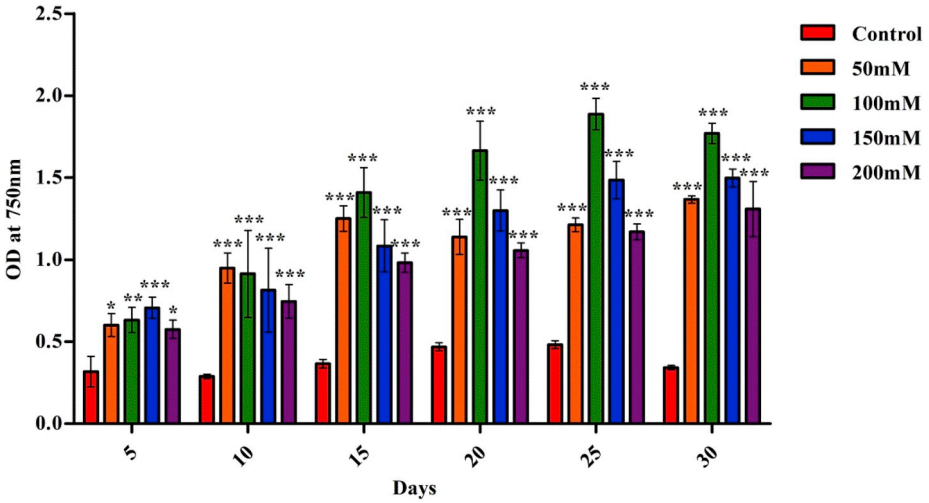

Fig. 1. Impact of Sodium bicarbonate on growth in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group. (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

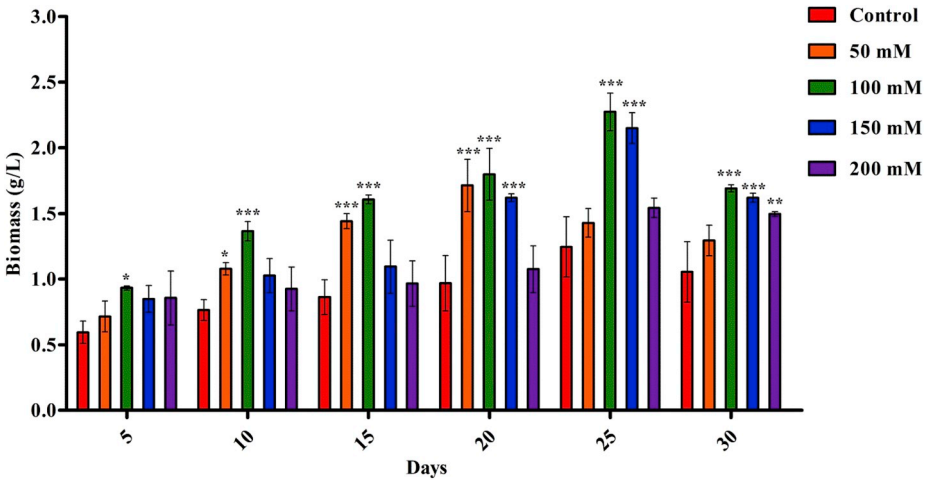

Fig. 2. Impact of Sodium bicarbonate on biomass in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group. (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

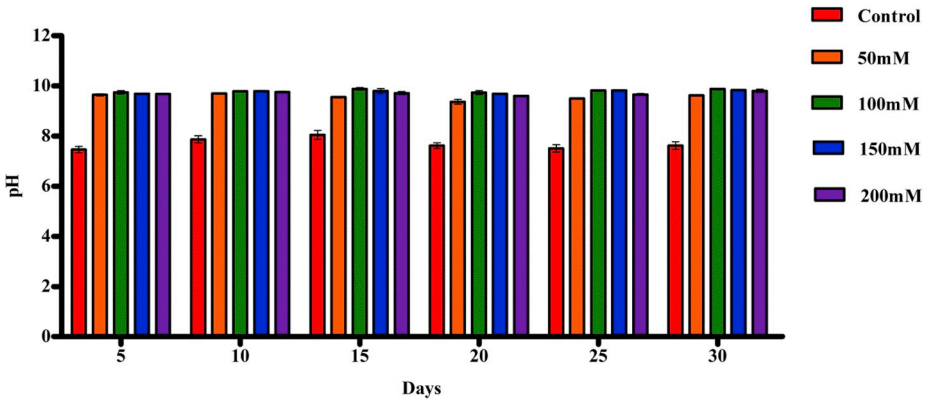

Fig. 3. pH variation during the growth of *Chlorella pyrenoidosa.* at various concentrations of sodium bicarbonate.
Values are expressed as mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group. (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

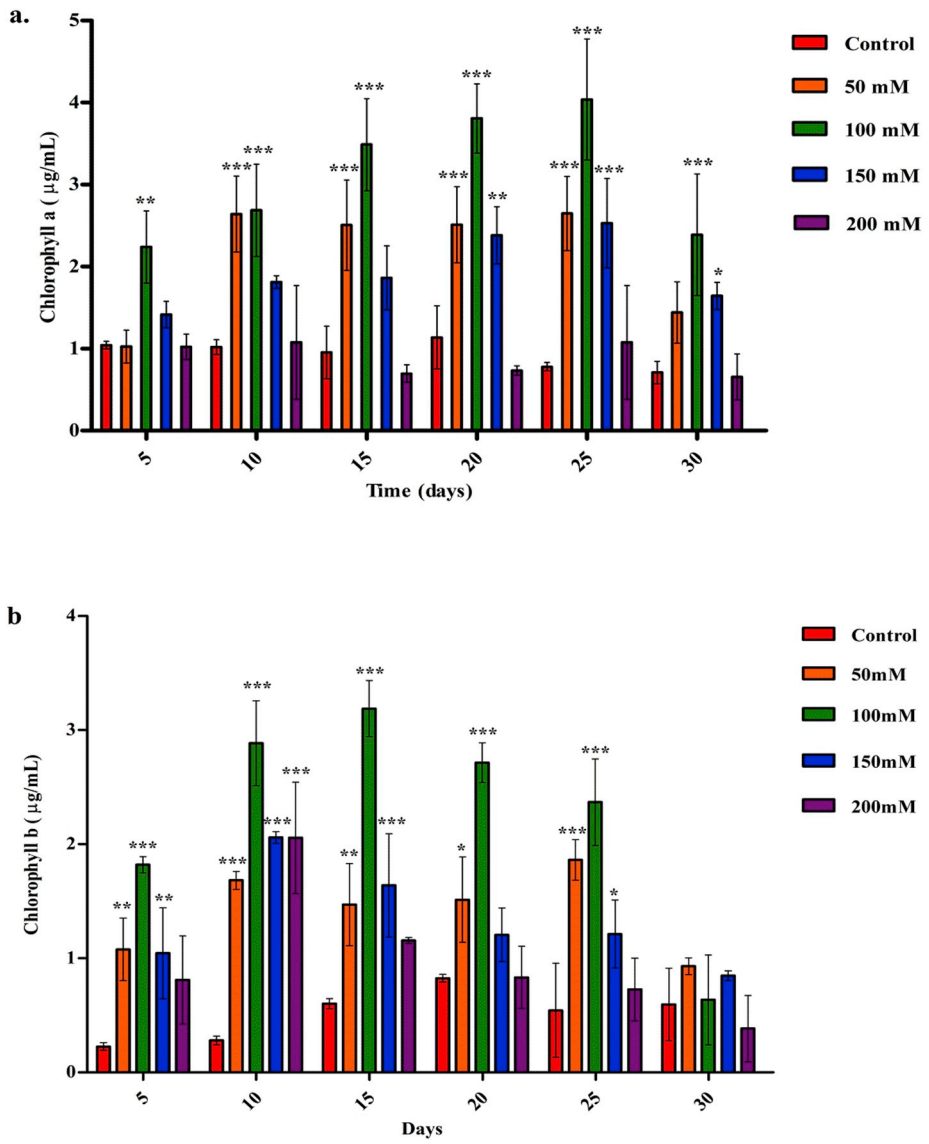

Fig. 4. Effect of sodium bicarbonate on (a) Chlorophyll *a* and (b) Chlorophyll *b* on *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group5 (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

control culture. The latter showed the highest chlorophyll content on day 20 and decreased gradually (Fig. 4). The total carotenoid content, measured every 5 days at 470 nm, was observed to be highest for 100 mM sodium bicarbonate concentration showed at day 30 with an yield of 5.77 ± 0.045 mg/g of DCW with respective to the control samples (1.83 ± 0.092 mg/g of DCW), the total carotenoid content in the other concentrations of sodium bicarbonate was found to increase from the 15th day to the 30th day (Fig. 5). Chlorophyll and carotenoids are the two major categories of intracellular pigments that are present in microalgae. Carotenoids are usually produced in very less quantity that the chlorophyll pigments in the cytoplast of the photosynthetic organisms. Photodynamic sensitization of chlorophyll pigments is prevented by coexistence of chlorophyll and carotenoids, whose absence can lead to the degradation of chloroplast (Goodwin, 1980). Both the chlorophyll pigments can be used as potential indicators of microalgae growth. Moreover, algal biomass can be indirectly quantified using chlorophyll content. In previous reports, when the microalgae cells are exposed to unfavorable conditions, such as high salt or nutrient-deficient conditions, it has been reported that there is a significant decrease in the chlorophyll content, an increase in the carotenoid production and also arrest in cell biomass production (Sampathkumar et al., 2019). Similar pattern of increase in the chlorophyll pigments (a and b) and total carotenoid levels were also observed in the present study. The chlorophyll pigments and total carotenoid levels were found to increase up to 100 mM and at concentration higher than 100 mM, the production of chlorophyll and carotenoid levels decreases.

3.3. Impact of Sodium bicarbonate supplementation on lutein production

Chlorella pyrenoidosa was grown in fog's media supplemented with different concentrations of sodium bicarbonate. The impact of supplementing sodium bicarbonate to the fog's media on lutein biosynthesis in *C. pyrenoidosa* was investigated using HPLC. Lutein production was found to increase significantly during day 30 in the cultures supplemented with sodium bicarbonate compared to control culture. Lutein production was found to have been enhanced in 100 mM concentration (4.84 ± 0.56 mg/g of DCW), followed by 50 mM concentration (4.21 ± 0.11 mg/g of DCW) supplemented cultures compared to 150 mM and 200 mM concentration (2.32 ± 0.093 mg/g of DCW and 2.31 ± 0.114 mg/g of DCW) and control culture (1.45 ± 0.18 mg/g of

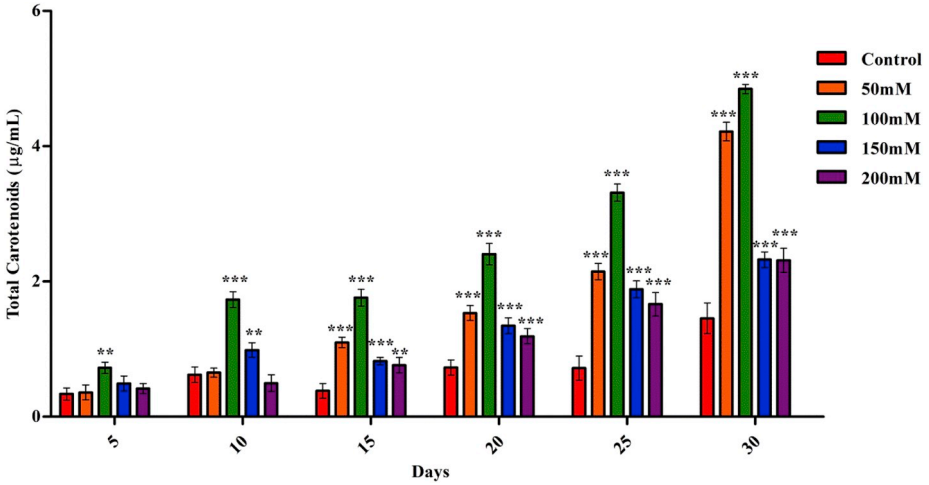

Fig. 5. Effect of sodium bicarbonate on total carotenoids in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

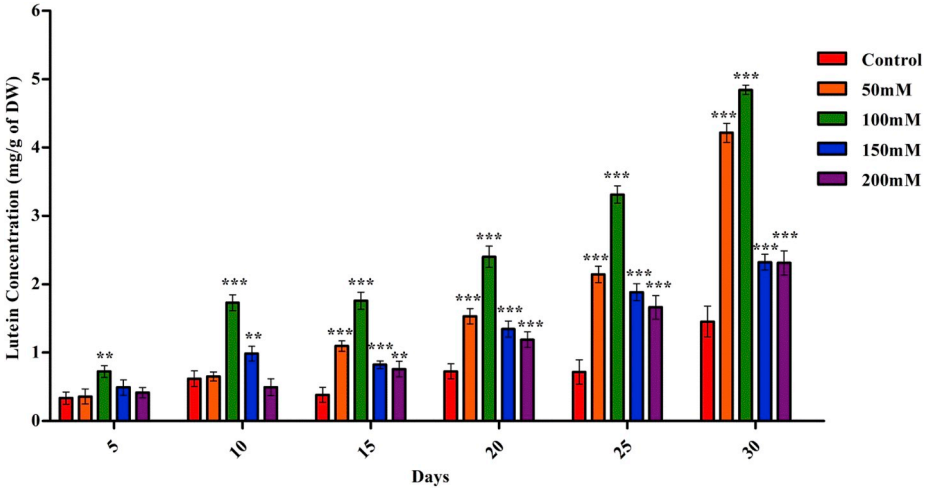

Fig. 6. Effect of sodium bicarbonate on lutein production in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

DCW) (Fig. 6). When *C. pyrenoidosa* is subjected to any modification in its media composition, it experiences oxidative stress due to the release of excessive ROS species. This characteristic of *C. pyrenoidosa* were observed in other microalgal species too, where, enhancement of lutein was observed when the microalgae were subjected to various abiotic stresses such as nutrient starvation (Azadeh et al., 2017; Sampathkumar et al., 2019), salt stress (Cordero et al., 2011; Raja et al., 2007), modification in light intensity and wavelength (Chan et al., 2013), etc. The enhancement and yield of lutein obtained in the present study is in corroboration with the results that was obtained in the previous studies.

3.4. Impact of Sodium bicarbonate supplementation on the expression of genes involved in carotenogenesis of lutein

The expression analysis of the three genes, pds, lyc and chy, was carried out on the day that had the highest lutein yield (day 30). The gene expression was found to upregulated in all the treatments and the maximum up regulation of the genes was observed at 100 mM NaHCO3 (pds by 5.8 times, lyc by 4.3 times and chy by 3.9 times) concentration followed by 150 mM concentration (pds by 4 times, lyc by 3.8 times and chy by 3.2 times). This shows that when the microalgal culture was subjected to stress, the genes in the carotenogenesis pathway is up regulated to counteract the damage caused due to oxidative stress (Fig. 7). The expressions of genes that are involved in the carotenogenesis of lutein were also studied. The results obtained showed that there was an up regulation in the genes (pds, lyc, chy), which corroborates with the previous studies (Vidhyavathi et al., 2009).

3.5. Impact of Sodium bicarbonate supplementation on lipid production

Quantification of lipid accumulation was carried at 5 day interval for 30 days. The lipid production was found to be highest between 50 to 150 mM concentrations of sodium bicarbonate. The lipid concentration was found to increase in all samples between days 20 and 30 (Fig. 8). The highest yield was obtained at 100 mM concentration of sodium bicarbonate on day 25 with a yield of 0.018 ± 0.009 g/mL. Nutrient availability determines the fatty acid and lipid biosynthesis in microalgae. Supplementation of bicarbonate has been reported to enhance lipid and carotenoid production and also cell division in microalgal species (Guihéneuf and Stengel, 2013; Lam and Lee, 2013; Mukund and Senthilkumar, 2013). In the current study, the lipid accumulation was observed to be enhanced in the microalgal culture that was

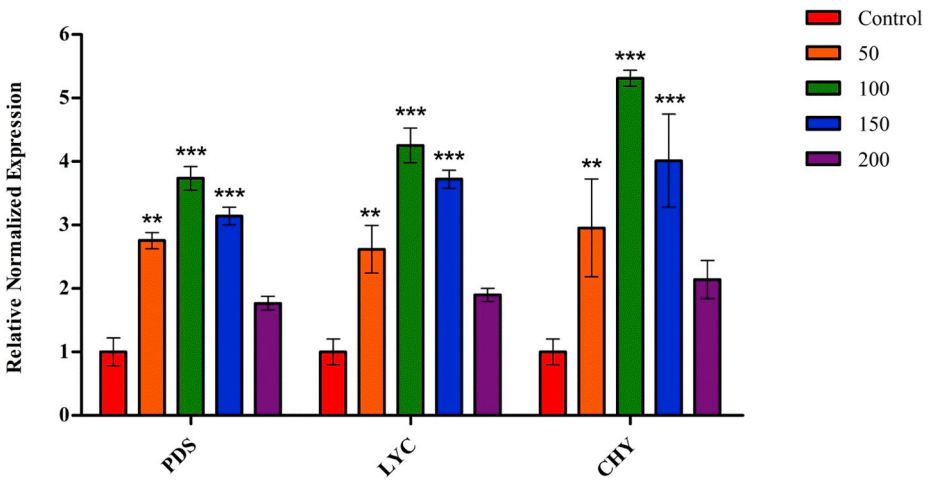

Fig. 7. Expression analysis of genes (pds, lyc, chy) involved in lutein carotenogenesis in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

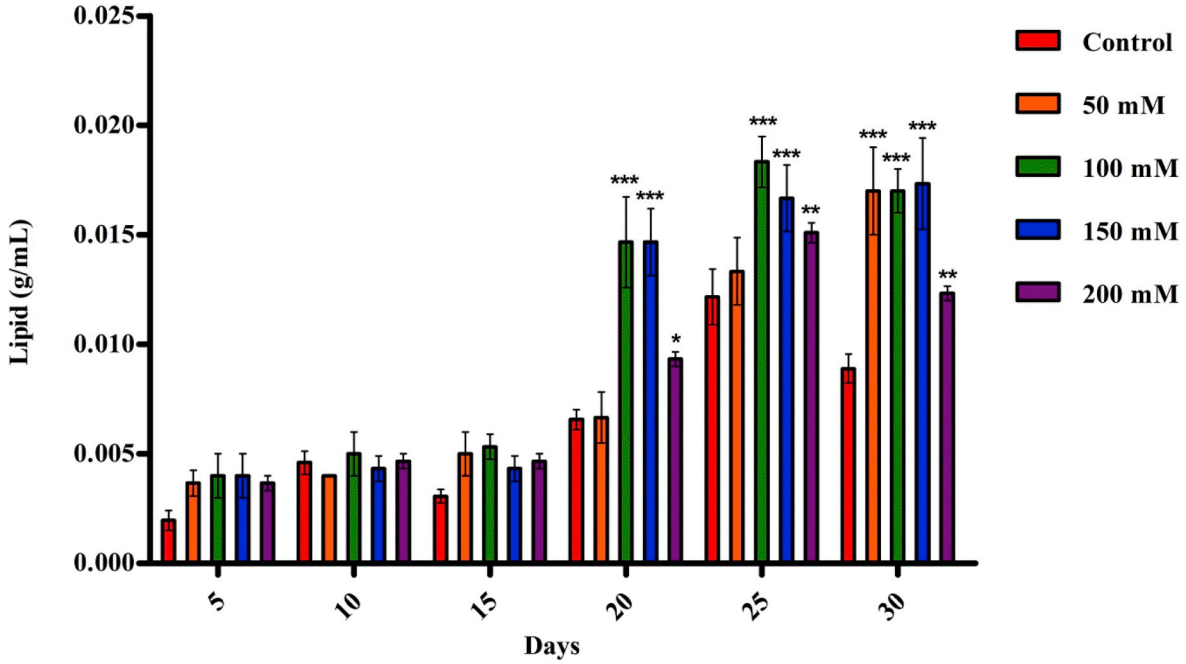

Fig. 8. Effect of sodium bicarbonate on lipid production in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

supplemented with bicarbonate, with a maximum yield of 0.018 ± 0.009 g/mL. These results were found to corroborate with previous studies (Chi et al., 2011; Takagi et al., 2000). The changes in the composition of fatty acids in microalgae and the accumulation of oils associated to fatty acid composition are controlled by normally under limitation or starvation condition, microalgae are the main key factors that control the accumulation of oil associated with changes in fatty acid composition (Lei et al., 2012; Sato et al., 2000; Yu et al., 2009).

3.6. Impact of Sodium bicarbonate supplementation on protein and SOD levels

The protein estimation was carried out at the end of the study period (day 30). The highest yield was obtained when the culture was treated with 100 mM sodium bicarbonate (5.95 ± 1.53 mg/g of DCW) followed by 150 mM (5.1 ± 0.61 mg/g of DCW), 200 mM (5.16 ± 0.12 mg/g of DCW), 50 mM (4.01 ± 0.82 mg/g of DCW) and control (2.18 ± 0.13 mg/g of DCW) cultures (Fig. 9). During any stress, the microalgal cell releases antioxidant enzymes to counter the reactive oxygen species produced. One of the major antioxidant enzyme that is produced is Superoxide Dismutase (SOD). The highest levels of SOD was observed in culture treated with 150 mM sodium bicarbonate (30 ± 2.94 nmol/g FW), followed by 200 mM (23.74 ± 0.89 nmol/g FW), 100 mM (17.66 ± 1.24 nmol/g FW), 50 mM (13.05 ± 0.65 nmol/g FW) and control (10.58 ± 1.04 nmol/g FW) (Fig. 10).

4. Conclusions

Over the past few years, algal biotechnology industry has been growing rapidly and biotechnological potentials of microalgae is being

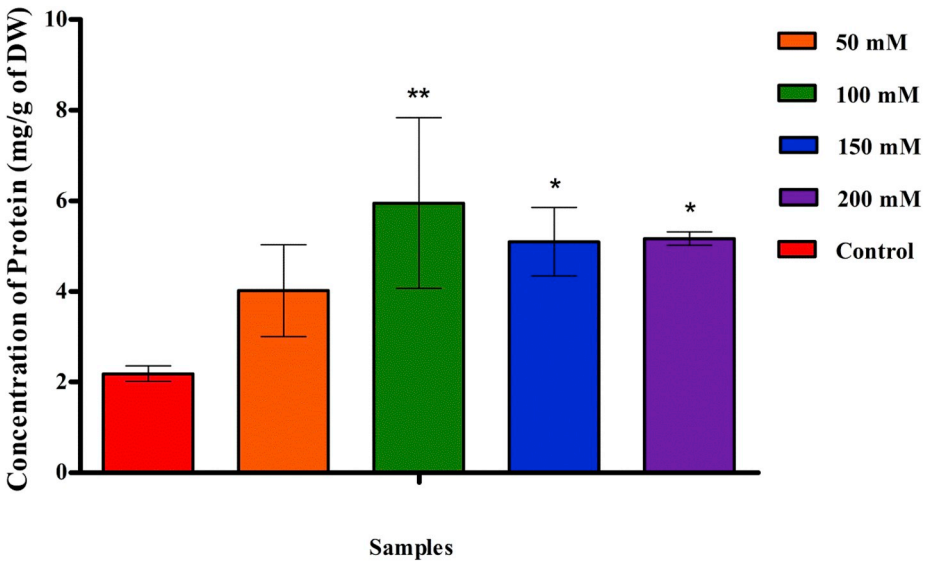

Fig. 9. Effect of sodium bicarbonate on protein in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

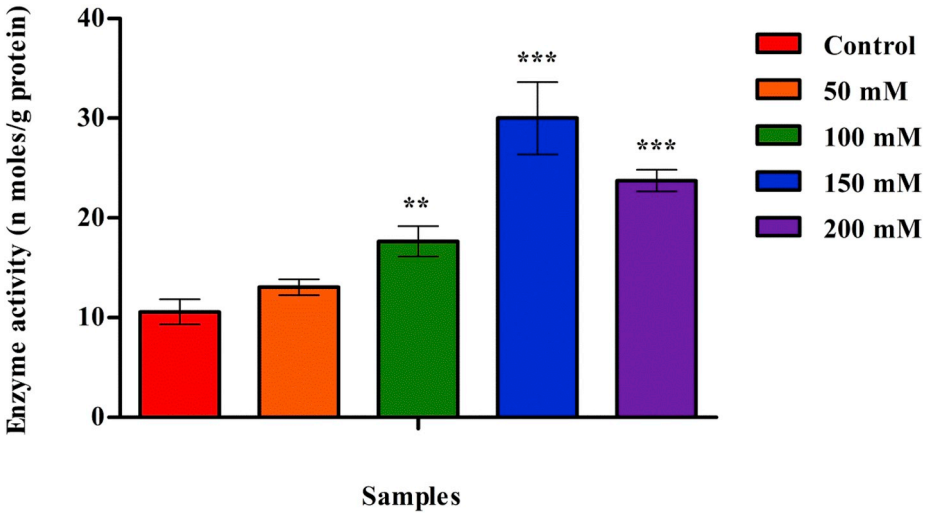

Fig. 10. Antioxidant activity of SOD during sodium bicarbonate supplementation in *C. pyrenoidosa*. All values are represented as Mean ± SD (n = 3). Values with different no. of * represents the significant difference at $p < 0.05$ between the treatment groups and the control group (Color image). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

studied for various applications. Microalgae are potential and superior candidate for carotenoid accumulation and lipid production. The microalga *Chlorella* is widely used and commercialized in variety of applications like food, fuel and products. Recent efforts on extraction and purification of lutein from microalgae have been growing but more systematic studies are needed to identify economical methods to produce maximum lutein from microalgal sources. In this article, enhancement of lutein and total lipid from *C. pyrenoidosa* grown in sodium bicarbonate supplemented medium is carried out. The results showed at three times increase in the lutein production during bicarbonate supplementation condition and the lipid yield was also enhanced significantly. From the present study finally it can be concluded that the optimum bicarbonate concentration for enhancement of fatty acid and lutein accumulation in *Chlorella pyrenoidosa* was in the range between 50 and 100 mM. Therefore, for improving the production of lipids and lutein for large scale applications, sodium bicarbonate can be taken into consideration as a suitable carbon source for *Chlorella* sp.

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Declaration of competing interest

The authors declare no conflict of interest regarding the publication of this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101406.

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