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Sodium Bicarbonate as Inorganic Carbon Source for Higher Biomass and Lipid Production Integrated Carbon Capture in *Chlorella vulgaris*

Keyuri Mokashi [a], Vishaka Shetty [b], Sangeetha Annie George [c], G. Sibi [b,*]

[a] Department of Genetics Indian Academy Degree College Centre for Research and Post Graduate Studies, Bengaluru - 560 043, Karnataka, India
[b] Department of Biotechnology, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bengaluru 560 043, Karnataka, India
[c] Department of Zoology Indian Academy Degree College Centre for Research and Post Graduate Studies, Bengaluru - 560 043, Karnataka, India

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ABSTRACT

Chlorella vulgaris was isolated from sewerage treatment plant and grown in the presence of sodium bicarbonate as carbon source at 0.25, 0.5 and 1.0 g L^{-1}. Highest specific growth rate (0.653 μd^{-1}) was obtained with 1 g L^{-1} bicarbonate followed by 0.5 g L^{-1} (0.641 d^{-1}) on 15th day culturing. Total chlorophyll content of microalgae has increased in a dose dependent fashion with bicarbonate addition and maximum level recorded in 1 g L^{-1} (0.769 ± 0.09 g L^{-1}). The biomass productivity was in the range of 0.237–0.996 g d^{-1} L^{-1}. Rate of CO_2 fixation and carbon content, in terms of quantity was estimated. Results showed that at 1 g L^{-1} sodium bicarbonate concentration, maximum CO_2 fixation (0.497 g/dry weight) and carbon content (0.69 g mL^{-1} day^{-1}) was found. Biomass concentration was significantly higher ($p < 0.05$) in cultures (1.54 g L^{-1}) supplemented with 1 g L^{-1} bicarbonate whereas there was no much difference in cellular lipid concentration (16 mg mL^{-1}). GC–MS analysis of fatty acids showed highest amounts of palmitic acid, myristic and stearic acid. In summary, the addition of sodium bicarbonate increases cellular abundance, chlorophyll content and to some extent in the case of lipid content in *C. vulgaris* integrated with CO_2 sequestration.

Introduction

Among the atmospheric pollutants, CO_2 contributes significantly to the greenhouse effect. Global warming attributed primarily to the elevated CO_2 level in the atmosphere has led to various CO_2 mitigation strategies. As chemical reaction based CO_2 capturing is relatively costly and energy consuming, it is necessary to develop cost effective and sustainable alternatives. Biological CO_2 mitigation leads to the production of biomass energy and is an alternative strategy of CO_2 fixation through photosynthesis (Kondili and Kaldellis, 2007; de Morais and Costa, 2007). Microalgae are more efficient than terrestrial plants in photosynthesis from ambient air and are important for the prevention of increase in atmospheric CO_2 concentration. It converts CO_2 into biomass energy and thus recycles CO_2 (Demirbas, 2004). Coupling of CO_2 sequestration with algal cultivation reduces the carbon footprint and sustainable environment.

Microalgae have drawn more attention as a promising source for the production of biodiesel because they possess high growth rate and provide lipid fraction for biofuel production. Microalgal growth and biochemical composition are governed by environmental

* Corresponding author at: Department of Biotechnology Indian Academy Degree College Centre for Research and Post Graduate Studies, Bengaluru - 560 043, Karnataka, India. Tel.: +91 99864 52875.
E-mail address: gsibii@gmail.com (G. Sibi).
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conditions (Guiheneuf et al., 2008; Pal et al., 2011). Sodium bicarbonate has been demonstrated to enhance lipid accumulation in both freshwater and marine microalgae (Gardner et al., 2012, 2013; White et al., 2013; Peng et al., 2014). Microalgae utilize bicarbonate as the external source of carbon for photosynthesis and derive CO_2 via the action of carbonic anhydrase (Dixon et al., 1987; Nimer et al., 1997; Bozzo et al., 2000). This work established the growth of *Chlorella vulgaris* in a medium where sodium bicarbonate serves as a source of inorganic carbon by assessing growth rate, chlorophyll content, biomass and lipid production in batch cultures. Further, rates of inorganic carbon concentration and CO_2 fixation were determined for CO_2 sequestration using bicarbonate.

Materials and Method

Sample Collection and Identification

Algal samples were collected from Bangalore Water Supply and Sewerage Board (BWSSB), Bengaluru (13°04′N, 77°58′E) and poured into a closed 250 mL bottle and exposed in sunlight for 3 weeks. The upper layer of the water was inoculated in agar plates enriched with BG11 medium containing 200 μg mL^{-1} ampicillin to control the growth of bacteria as the sample used was sewage water. Agar plating technique was used to isolate the microalgae and the plates were incubated at 25 ± 2 °C under cool white fluorescent light (40 μmol photons m^{-2} s^{-1}; 15 h light/9 h dark) until algal growth was detected. The isolates were purified by streak plating and individual colonies were diluted in distilled water. Species of single cells were obtained using capillary pipette under a microscope followed by inoculation into fresh media. After appropriate growth, cells were observed to confirm the single culture and the capillary method was repeated as many times as required to obtain axenic cultures. Identification of *Chlorella* was using standard protocols as described by Anderson (2005), Stanier et al. (1971) and the database http://web.biosci.utexas.edu/utex/default.

Growth Under Different Concentrations of Sodium Bicarbonate

Analytical grade sodium bicarbonate was used as the source of bicarbonate in all experiments. Batch cultures (100 mL) of *C. vulgaris* (BG 11 medium; n = 3) were grown under different levels of bicarbonate supplementation (0.25, 0.5 and 1 g L^{-1}) into early stationary growth phase (10–15 days), where samples were taken for growth rate, biomass productivity, chlorophyll content and cellular lipid analyses. Media without the addition of bicarbonate were served as control.

Analytical Methods

Biomass Concentration and Productivity

Biomass concentration (g L^{-1}) of *C. vulgaris* grown under different bicarbonate concentrations was determined by measuring the optical density (OD_{680}) using UV–Vis spectrophotometer. The result was converted to biomass concentration using the calibration curve relating OD_{680} (Xia et al., 2014) using the following Eq. (1)

$$\text{Biomass concentration} = 320 \times OD_{680}. \tag{1}$$

The biomass productivity (mg L^{-1} d^{-1}) was calculated according to Eq. (2).

$$\text{Biomass productivity} = (B_2 - B_1)/T \tag{2}$$

where B_2 and B_1 represent the dry weight biomass densities at the time T (days), at the end and start of the experiment, respectively.

Specific Growth Rate

Specific growth rate (μ) of the microalgae was calculated (Guillard and Ryther, 1962) according to the following formula.

$$\mu = \frac{\ln(N_t/N_0)}{T_t - T_0.}$$

where, N_t and N_0 are the total cells at the end of log phase (T_t) and start of log phase (T_0), respectively.

Chlorophyll Estimation

Chlorophyll a and b of microalgae were estimated according to Mackinney (1941). Algal suspension was filtered and extracted with methanol in water bath at 60 °C for 30 min. The suspension was cooled, added equal volume of 96% methanol and centrifuged for 6500 *g* for 10 min. Pigment content of the supernatant was analyzed in a UV–Vis spectrophotometer at 650 nm and 665 nm using 96% methanol as blank and the total chlorophyll was determined using Eq. (3)

$$\text{Total chlorophyll} = 2.55 \times 10^{-2}.E650 + 0.4 \times 10^{-2}.E665 \text{ mg ml}^{-1} \tag{3}$$

where, E650 and E665 are the absorbance at 650 and 665 nm wavelengths respectively.

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Estimation of Carbon Content and Carbon Dioxide Fixation Rate

Carbon content of the dried algal biomass was estimated by Walkley and Black (1934). Dried algal sample was mixed with 1 N potassium dichromate (10 mL) and conc. H_2SO_4 (20 mL) mixture diluted with distilled water (200 mL) followed by addition of H_3PO_4 (10 mL) and diphenyl amine (1 mL). The reaction mixture was titrated against 4 N ferrous ammonium sulfate (FAS) until the appearance of brilliant green color and the carbon content was estimated using Eq. (4).

$$a = \frac{3.951}{g}\left(1 - \frac{T}{S}\right) \tag{4}$$

where, a is the carbon content, g is the weight of algal sample, T and S are the ferrous ammonium sulfate with blank and sample (mL), respectively.

The amount of carbon dioxide fixation rate was estimated using Eq. (5). (Yun et al., 1997)

$$R_{CO_2} = C_C \times \mu_L \left({}^{M_{CO_2}}\Big/_{M_C}\right) \tag{5}$$

where, R_{CO2} and μL are the CO_2 fixation rate (g CO_2 m^{-3} h^{-1}) and the volumetric growth rate (g dry weight m^{-3} h^{-1}), respectively, C_C is the average carbon content (algal dry weight [g]). M_{CO2} and M_C are the molecular weights of CO_2 and elemental carbon, respectively.

Lipid Extraction

Algal cultures were pelleted and added with 10 mL of ice cold 0.2 N $HClO_4$. After 15 min at 4 °C, the sample was centrifuged and the supernatant was added with 10 mL of 0.2 N $HClO_4$ followed by centrifugation. To the pellet, 10 mL of chloroform–methanol (2:1) solution was added, centrifuged and 0.2 volumes of distilled water were added to the supernatant. The mixture was centrifuged and the lower organic phase was collected, evaporated under a stream of nitrogen to a final volume of 2 mL (Folch et al., 1956).

Lipid Estimation

Standard solutions of palmitic acid (5, 10, 20 and 30 mg mL^{-1}) and algal lipid samples were prepared in chloroform, added with 2 mL of dichromate solution (2.5 g of $K_2Cr_2O_7$ in 1 L of conc. H_2SO_4) and placed in a boiling water bath for 45 min. The mixture was cooled, removed 1 mL from each and diluted to 100 mL with distilled water followed by measuring the absorbance at 350 nm (Marsh and Weinstein, 1966).

Fatty Acid Methyl Ester Preparation

The lipid extract was saponified with 5 mL of 0.5 N methanolic solution of potassium hydroxide by boiling for 3–5 min (Hartman and Lago, 1973). To this, 15 mL of esterification reagent (2 g of ammonium chloride in 60 mL of methanol and 3 mL of conc. H_2SO_4) was added and refluxed for 3 min. Subsequently, it was transferred to a separating funnel using 25 mL of diethyl ether and 50 mL of saturated sodium chloride solution and the aqueous layer discarded. The diethyl ether layer was washed twice with 25 mL of NaCl solution and the solvent was evaporated under a stream of nitrogen for GC–MS analysis.

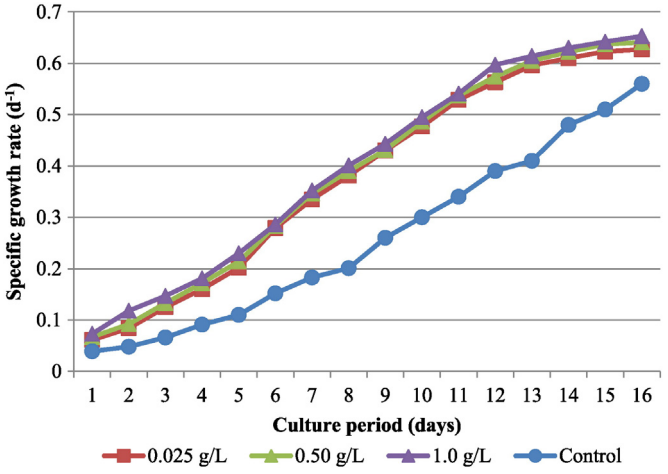

Fig. 1. Specific growth rate of *C. vulgaris* culture supplemented with different levels of bicarbonate.

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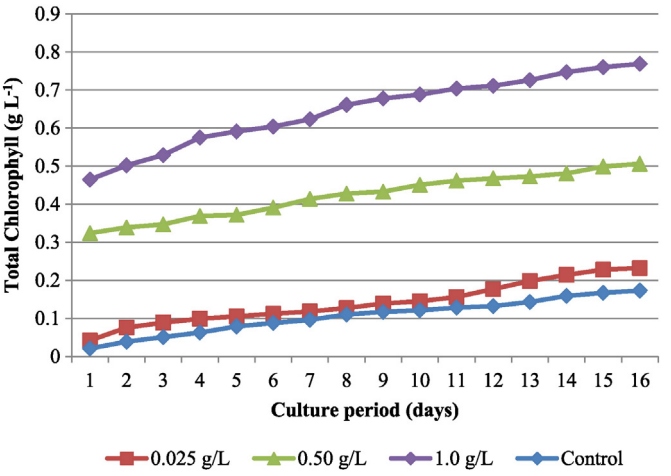

Fig. 2. Chlorophyll content of *C. vulgaris* culture supplemented with different levels of bicarbonate.

GC–MS Analysis

GC–MS analysis of lipid extract was performed using an Agilent GC comprising a Thermo DSQII auto-sampler equipped with a DB 5 MS column (30 m × 0.25 mm ID × 0.25 μm). For GC–MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1.0 mL min^{-1} and an injection volume of 1 μL was employed in a split mode (1:10). The initial temperature was 40 °C with a hold time of 2 min and the ramp was 310 °C at a rate of 10 °C/min for a hold period of 10 min. Mass spectra were taken at a scan mass range of 30–600 m/z. The solvent delay was 0 to 2 min and the total GC/MS running time was 32 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Quadrupole mass analyzer along with Xcalibur and AMDIS software was adopted to handle mass spectra and chromatograms were compared with NIST 2011.

Statistical Analysis

All the experiments were carried out in triplicates and the results were expressed as mean values and the standard deviation (SD). The statistical differences were obtained through one-way analysis of variance (ANOVA) ($p < 0.05$).

Results and Discussion

The effect of bicarbonate concentration on specific growth rate (μ) of *C. vulgaris* is shown in Fig. 1. Specific growth rate was not significantly different within the concentrations of $NaHCO_3$. It showed a similar trend in growth rate with 1 g L^{-1} bicarbonate maintaining the highest specific growth rate (0.653 d^{-1}) at the end of 15 days culture period followed by 0.5 g L^{-1} (0.641 d^{-1}).

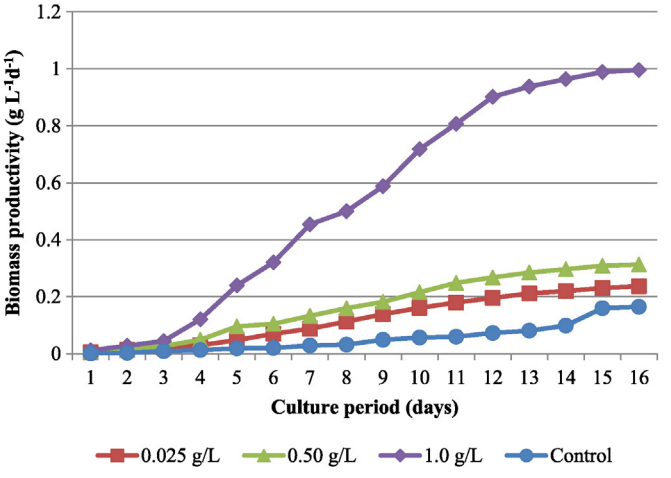

Fig. 3. Biomass productivity of *C. vulgaris* culture supplemented with different levels of bicarbonate.

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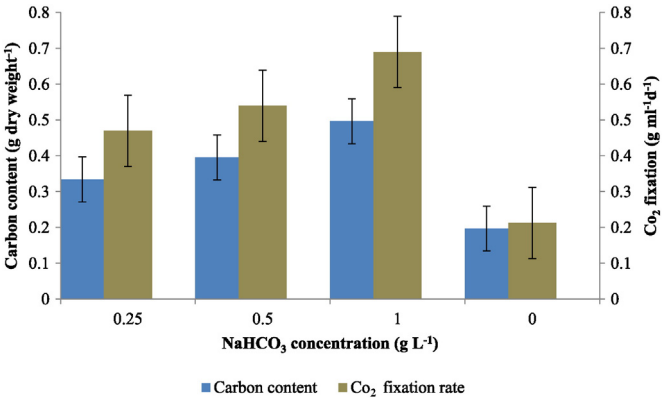

Fig. 4. Carbon content and CO_2 fixation rate in *C. vulgaris* supplemented with different levels of bicarbonate.

Significant positive effect on the levels of chlorophyll content with an approximate doubling or more with the addition of 1 g L^{-1} bicarbonate when compared to treatments received 0.25 g L^{-1} and 0.5 g L^{-1}(Fig. 2) was observed. At the same time, both Chl a and b were significantly varied between lower concentrations of bicarbonate. The total chlorophyll content increased in a dose dependent fashion with bicarbonate addition and maximum level recorded in 1 g L^{-1} (0.769 ± 0.09 g L^{-1}).

The first equation gives the biomass concentration of the culture. In the 2nd equation, B1 is the early stationary phase and B2 is the end of stationary phase. Stationary phase was considered rather than exponential, as the data is valid at the end of stationary phase in terms of biomass productivity. The addition of bicarbonate had significant effects on the biomass productivity in *C. vulgaris* ranged from 0.237–0.996 g d^{-1} L^{-1}. As depicted in Fig. 3, biomass productivity at 1 g L^{-1} (0.996 ± 0.06 g d^{-1} L^{-1}) was significantly higher ($p < 0.05$) than those cultures with lower bicarbonate addition. Reduced biomass productivity was observed with lower concentrations of sodium bi carbonate throughout the culturing period.

Carbon content of the dried algal biomass was estimated and increasing carbon content enhanced the CO_2 fixation. At 1 g L^{-1} sodium bicarbonate concentration, carbon content was maximum (0.497 g dw^{-1}) and CO_2 fixation was 0.69 g mL^{-1} day^{-1}. The lowest level of CO_2 fixation was observed in 0.25 g L^{-1} bicarbonate addition (Fig. 4).

The results of biomass concentration and lipid production at the end of lag phase are compared in Fig. 5. At 1 g L^{-1} of sodium bicarbonate levels, biomass concentration was significantly higher (1.542 g L^{-1}) which was triple the amount obtained at 0.5 g L^{-1} bicarbonate addition. However, there was no much difference in lipid production at varying bicarbonate concentrations. Cellular lipid concentration was determined and the total cellular lipid was highest in cultures supplemented with 1 g L^{-1} bicarbonate (16.1 ± 0.07 mg mL^{-1}) compared to cultures with 0.5 and 0.25 g L^{-1} bicarbonate (14.3 ± 0.14 and 12.4 ± 0.21 mg mL^{-1}). Based on highest total lipid production, fatty acid profiles in *C. vulgaris* grown under 1.0 g L^{-1} bicarbonate addition were determined post-conversion to fatty acid methyl esters by GC–MS (Fig. 6). Sodium bicarbonate has promoted the fatty acid profiles significantly and the key fatty acids were palmitic, myristic and stearic acid alongside several minor fatty acid components with a carbon number of C16 and C18.

Cultivation conditions can maximize the algal biomass production rates and lipid concentrations. Carbon limitations due to the poor solubility of CO_2 in water can lead to growth inhibition (Smith and Bidwell, 1989; Giordano et al., 2005; Aishvarya et al., 2012). $NaHCO_3$ has greater solubility than CO_2 and could be an alternative inorganic carbon source (Hsueh et al., 2007) for microalgal cultivation. Further, gaseous CO_2 is costly to store and transport, whereas bicarbonate salts can easily be transported to algal facilities and stored until needed. Enhanced inorganic carbon uptake due to the addition of bicarbonate allows cellular material production and

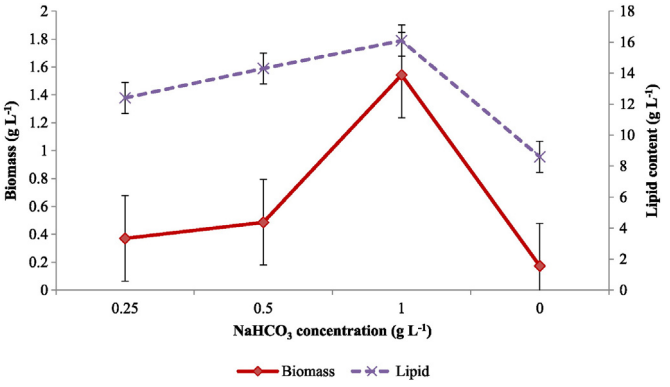

Fig. 5. Biomass and cellular lipid production in *C. vulgaris* supplemented with different levels of bicarbonate.

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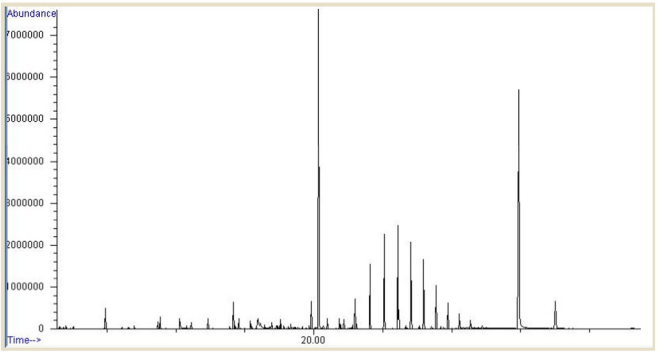

Fig. 6. GC–MS profile of fatty acid methyl esters from *C. vulgaris* at 1.0 g L^{-1} bicarbonate concentration.

thereby achieving maximum productivity. In this study, maximum biomass of 0.996 g L^{-1} d^{-1} was observed in 1 g L^{-1} sodium bicarbonate addition and this is in agreement with Yeh et al. (2010) who have demonstrated that 1 g L^{-1} sodium bicarbonate was found optimum for biomass production in *C. vulgaris*. In another study, 1 M $NaHCO_3$ has produced higher biomass concentration (Chi et al., 2013).

Conventionally, chlorophyll is considered as a reliable and standard algal biomass measurement (Wiltshire et al., 1998; Knefelkamp et al., 2007). Physiological state of microalgae is indicated by carbon to chlorophyll ratio and in this study total chlorophyll content was taken as the indicator of physiology of *C. vulgaris* influenced by inorganic carbon (sodium bicarbonate). Total chlorophyll content of 0.769 g L^{-1} was determined in cultures grown in the highest concentration of bicarbonate and was lower in decreasing concentrations. Higher chlorophyll biosynthesis was observed in the presence of sodium bicarbonate at 1 g L^{-1} in *C. vulgaris* (Kong et al., 2011) where as it was 0.005 M in *Chlorella salina* (Jayasankar and Valsala, 2008). Growth rate of microalgal population is another measure of increase in biomass and increase in specific growth rate was observed at 1 g L^{-1} over time. The duration of exponential phase in terms of specific growth rate was longer which explains impact of sodium bicarbonate in the culture medium. It was clear that higher photosynthetic efficiency due to increase in chlorophyll content indicates sodium bicarbonate would promote the growth rate as well as biomass production. Biofixation of CO_2 using microalgae and converting it into biomass by way of photosynthesis is a promising way to sequester CO_2 (Watanabe and Hall, 1996; Ono and Cuello, 2006; Ryu et al., 2009). High CO_2 tolerant *Chlorella* sp. with good growth rate was reported (Sung et al., 1998, 1999; Yue and Chen, 2005).The amount of carbon content and CO_2 fixation rates were determined in *C. vulgaris* and carbon content in the range of 0.334–0.497 g dry weight^{-1} was obtained. Similar carbon content in *C. vulgaris* was reported in earlier studies (Yun et al., 1997; Pandian and Ravindran, 2012). It was observed that at higher bicarbonate levels, increased carbon sequestration was found.

Bicarbonate induces carbon storage metabolic activity and triggers lipid accumulation (Gardner et al., 2012, 2013; White et al., 2013). Higher neutral lipid production in microalgae with the addition of bicarbonate was reported by Bywaters and Fritsen (2015). Sodium bicarbonate has been used as a carbon source for the study of growth and biochemical composition in a range of microalgae species (Guiheneuf et al., 2008, 2009; Sostaric et al., 2009; Pimolrat et al., 2010; Yeh et al., 2010) and has been shown to stimulate triacylglycerol accumulation in microalgal species. The length of carbon chain and the number of double bonds in fatty acids affect its fuel properties in which C16:1 and C18:1 are the ideal biodiesel feedstock (Knothe, 2008; Stansell et al., 2012). One of the main objectives of this study is to enhance lipid content of microalgae for biodiesel production hence the lipid class which are suitable for biodiesel production was considered. In this study, triglycerides with carbon chain lengths of C14–C17 were obtained revealing the potential of bicarbonate addition in microalgal cultivation for biofuel production. It was noted that although increased bicarbonate concentration has increased lipid productivity, the values were still in the lower range of those reported for selected species.

Conclusion

This study has the potential to aid industrial algal biofuel production with the use of sodium bicarbonate as an alternative inorganic carbon source to trigger biomass and lipid production. From the results, it was clear that bicarbonate addition had significant effects on pigment, biomass and lipid production in *C. vulgaris* isolated from sewage treatment plant. Microalgae exposed to 1 g L^{-1} concentration of bicarbonate expressed three times higher biomass concentration compared to other treatments. Further, the amount of total chlorophyll content, CO_2 fixation, biomass and lipid productivity were significantly influenced by the addition of sodium bicarbonate.

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References

Aishvarya, V., Pradhan, N., Nayak, R.R., Sukla, L.B., Mishra, B.K., 2012. Enhanced inorganic carbon uptake by *Chlorella* sp. IMMTCC-2 under autotrophic conditions for lipid production and CO_2 sequestration. J. Appl. Phycol. 24, 1455–1463.

Anderson, R.A. (Ed.), 2005. Algal Culturing Techniques. Elsevier Inc., USA.

Bozzo, G.G., Colman, B., Matsuda, Y., 2000. Active transport of CO_2 and bicarbonate is induced in response to external CO_2 concentration in the green alga *Chlorella kessleri*. J. Exp. Bot. 51, 1341–1348.

Bywaters, K.F., Fritsen, C.H., 2015. Biomass and neutral lipid production in geothermal microalgal consortia. Front. Bioeng. Biotechnol. 2, 1–11.

Chi, Z., Xie, Y., Elloy, F., Zheng, Y., Hu, Y., Chen, S., 2013. Bicarbonate-based integrated carbon capture and algae production system with alkalihalophilic cyanobacterium. Bioresour. Technol. 1333, 513–521.

de Morais, M.G., Costa, J.A.V., 2007. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers. Manag. 48, 2169–2173.

Demirbas, A., 2004. Current technologies for the thermo conversion of biomass into fuels and chemicals. Energy Sources 26, 715–730.

Dixon, G.K., Patel, B.N., Merrett, M.J., 1987. Role of intracellular carbonic anhydrase in inorganic-carbon assimilation by *Porphiridium purpureum*. Planta 172, 508–513.

Folch, J., Lees, M., Stanley, G.H.S., 1956. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Gardner, R.D., Cooksey, K.E., Mus, F., Macur, R., Moll, K., Eustance, E., Carlson, R.P., Gerlach, R., Fields, M.W., Peyton, B.M., 2012. Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the chlorophyte *Scenedesmus* sp. and the diatom *Phaeodactylum tricornutum*. J. Appl. Phycol. 24, 1311–1320.

Gardner, R.D., Lohman, E., Gerlach, R., Cooksey, K.E., Peyton, B.M., 2013. Comparison of CO_2 and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. Biotechnol. Bioeng. 110, 87–96.

Giordano, M., Beardall, J., Raven, J.A., 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu. Rev. Plant Biol. 56, 99–131.

Guiheneuf, F., Mimouni, V., Ulmann, L., Tremblin, G., 2008. Environmental factors affecting growth and omega 3 fatty acid composition in *Skeletonema costatum*. The influences of irradiance and carbon source. Diatom Res. 23, 93–103.

Guiheneuf, F., Mimouni, V., Ulmann, L., Tremblin, G., 2009. Combined effects of irradiance level and carbon source on fatty acid and lipid class composition in the *microalga Pavlova lutheri* commonly used in mariculture. J. Exp. Mar. Biol. Ecol. 369, 136–143.

Guillard, R.R.L., Ryther, J.H., 1962. Studies on marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8, 229–239.

Hartman, L., Lago, R.C.A., 1973. Rapid preparation of fatty acid methyl esters from lipids. Lab. Pract. 22, 475–476.

Hsueh, H.T., Chu, H., Yu, S.T., 2007. A batch study on the biofixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. Chemosphere 66, 878–886.

Jayasankar, R., Valsala, K.K., 2008. Influence of different concentrations of sodium bicarbonate on growth rate and chlorophyll content of *Chlorella salina*. J. Mar. Biol. Assoc. India 50, 74–78.

Knefelkamp, B., Carstens, K., Wiltshire, K.H., 2007. Comparison of different filter types on chlorophyll-a retention and nutrient measurements. J. Exp. Mar. Biol. Ecol. 345, 61–70.

Knothe, G., 2008. "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy Fuel 22, 1358–1364.

Kondili, E.M., Kaldellis, J.K., 2007. Biofuel implementation in East Europe: current status and future prospects. Renew. Sustain. Energy Rev. 11, 2137–2151.

Kong, W., Song, H., Cao, Y., Yang, H., Hua, A., Xia, C., 2011. The characteristics of biomass production, lipid accumulation and chlorophyll biosynthesis of *Chlorella vulgaris* under mixotrophic cultivation. Afr. J. Biotechnol. 10, 11620–11630.

Mackinney, J., 1941. Absorption of light by chlorophyll. J. Biol. Chem. 140, 315–322.

Marsh, J.B., Weinstein, D.B., 1966. Simple charring method for determination of lipids. J. Lipid Res. 7, 574–576.

Nimer, N.A., Iglesias-Rodriguez, M.D., Merrett, M.J., 1997. Bicarbonate utilization by marine phytoplankton species. J. Phycol. 33, 625–631.

Ono, E., Cuello, J.L., 2006. Feasibility assessment of microalgal carbon dioxide sequestration technology with photobioreactor and solar collector. Biosyst. Eng. 95, 597–606.

Pal, D., Khozin-Goldberg, I., Cohen, Z., Boussiba, S., 2011. The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp. Appl. Microbiol. Biotechnol. 90, 1429–1441.

Pandian, P., Ravindran, A.D., 2012. Lipid extraction and CO_2 mitigation by microalgae. J. Biochem. Technol. 4, 469–472.

Peng, X., Liu, S., Zhang, W., Zhao, Y., Chen, L., Wang, H., Liu, T., 2014. Triacylglycerol accumulation of *Phaeodactylum tricornutum* with different supply of inorganic carbon. J. Appl. Phycol. 26, 131–139.

Pimolrat, P., Direkbusarakom, S., Chinajariyawong, C., Powtongsook, S., 2010. The effect of sodium bicarbonate concentrations on growth and biochemical composition of *Chaetoceros gracilis* Schutt. Kasetsart Univ. Fish. Res. Bull. 34, 40–47.

Ryu, H.J., Oh, K.K., Kim, Y.S., 2009. Optimization of the influential factors for the improvement of CO_2 utilization efficiency and CO_2 mass transfer rate. J. Ind. Eng. Chem. 15, 471–475.

Smith, R.G., Bidwell, R., 1989. Mechanism of photosynthetic carbondioxide uptake by the red macroalga, *Chondrus crispus*. Plant Physiol. 89, 93–99.

Sostaric, M., Golob, J., Bricelj, M., Klinar, D., Pivec, A., 2009. Studies on the growth of *Chlorella vulgaris* in culture media with different carbon sources. Chem. Biochem. Eng. Q. 23, 471–477.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35, 171–205.

Stansell, R.G., Gray, M.G., Stuart, D.S., 2012. Microalgal fatty acid composition: implications for biodiesel quality. J. Appl. Phycol. 24, 791–801.

Sung, K.D., Lee, J.S., Shin, C.S., Park, S.C., 1998. Isolation of a new highly CO_2 tolerant fresh water microalga *Chlorella* sp. KR-1. Korean J. Chem. Eng. 15, 449–450.

Sung, K.D., Lee, J.S., Shin, C.S., Park, S.C., Choi, M.J., 1999. CO_2 fixation by *Chlorella* sp. KR-1 and its cultural characteristics. Bioresour. Technol. 68, 269–273.

Walkley, A., Black, I.A., 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–37.

Watanabe, Y., Hall, D.O., 1996. Photosynthetic CO_2 conversion technologies using a photobioreactor incorporating microalgae - energy and material balances. Energy Conserv. Manag. 37, 1321–1326.

White, D.A., Pagarette, A., Rooks, P., Ali, S.T., 2013. The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. J. Appl. Phycol. 25, 153–165.

Wiltshire, K.H., Harsdorf, S., Smidt, B., Blocker, G., Reuter, R., Schroeder, F., 1998. The determination of algal biomass (as chlorophyll) in suspended matter from the Elbe estuary and the German Bight: a comparison of high-performance liquid chromatography, delayed fluorescence and prompt fluorescence methods. J. Exp. Mar. Biol. Ecol. 222, 113–131.

Xia, L., Rong, J., Yang, H., He, Q., Zhang, D., Hu, C., 2014. NaCl as an effective inducer for lipid accumulation in freshwater microalgae *Desmodesmus abundans*. Bioresour. Technol. 161, 402–409.

Yeh, K.L., Chang, J.S., Chen, W.M., 2010. Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated microalga *Chlorella vulgaris* ESP-31. Eng. Life Sci. 10, 201–208.

Yue, L., Chen, W., 2005. Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae. Energy Convers. Manag. 46, 1868–1876.

Yun, Y.S., Lee, B.S., Park, T.M., Lee, C., Yang, J.W., 1997. Carbon dioxide fixation by algal cultivation using waste water nutrients. J. Chem. Technol. Biotechnol. 69, 451–455.

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