Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Influence of different concentrations of sodium bicarbonate on growth rate and biochemical composition of micro algae

Jegan, G, S.Mukund, N.S.Senthilkumar and RamaRajaVallinayagam
Department of Botany, RKM Vivekananda College, Chennai 600004, India

Abstract

Growth studies were conducted on micro green algae *Chlorella vulgaris*, *Chlorococcum humicola* and *Desmococcus olivaceous* in batch culture mode. In this study, the effect of sodium bicarbonate ($NaHCO_3$) as carbon source on micro algal cultures was investigated. All the strains showed increased growth and productivity in terms of increase in cell number, biomass and lipid content under increased bicarbonate utilization as carbon source. *Chlorella vulgaris* showed highest growth rate and lipid content.

Key words: Microalgae, bicarbonate, Carbon dioxide, biomass, lipid.

Introduction

Inorganic carbon (C_1) dissolved in seawater is mostly composed of high concentration of bicarbonate ion and low concentration of carbon dioxide (Israel and Gonzalez, 1996). The micro algae 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 plasma lemma (Dixon *et al.,* 1987) or externally allowing only bicarbonate to diffuse into the cell (Badges *et al.,* 1980). Photosynthesis in micro algae 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 micro algae operate by accumulating inorganic carbon intracellularly and the uptake is driven by energy coupled Ci transport system (Yingjun and Martin 2006).

In this present investigation an attempt was made to study the bicarbonate tolerance by selected micro algae. The growth rate and total carbohydrate, total protein, total lipid, β-carotene and chlorophyll a and b were determined in response to increased bicarbonate levels.

Materials and Methods

In our study three green algae, *Chlorella vulgaris*, *Chlorococcum humicola* and *Desmococcus olivaceous* were selected. The strains were obtained from the Culture Collection of Vivekananda Institute of Algal Technology (VIAT), Chennai.

Preparation of inoculums

The inoculums of the algal cultures used were prepared under laboratory condition. Micro algae were grown in Bold Basal medium at 24 ± 1^0C in a thermo-statically controlled room and illuminated with cool white inflorescence lamps (Philips 40W, Cool daylight 6500K) at an intensity of 2000 lux in a 12 hr: light dark regime. Sodium bicarbonate ($NaHCO_3$) was dissolved in BBM in different molar concentrations in which micro algae were inoculated. Light

intensity during the trials was measured using lux meter (lutron LX –101A). The micro algal cultures were microscopically examined using Olympus (HB) microscope and photomicrograph using Nikon digital camera (Coolpix E8400).

Growth measurement

Growth was measured by counting cells using a haemocytometer (Neubauer, improved) and the results were plotted in a semi-logarithmic graph. For dry weight method, the algal cultures were pelleted by centrifugation at 7500 rpm (Remi cooling microfuge) for 15 minutes. Cells were washed with glass-distilled water, again centrifuged and dried in an oven for 24 hours or until constant weight.

Estimation of Chlorophyll 'a and b' and β-Carotene

Chlorophyll 'a and b' (Jeffery and Humphrey (1975)) and β-carotene (Shaish *et al.*, 1992) were determined spectrophotometrically.

Estimation of Total Carbohydrates, Protein, Lipids.

The macromolecules such as carbohydrates (Pons *et al.*, 1981), protein (Lowry *et al.*, 1951) lipid (Bligh and Dyer *et al.*, 1959) were determined spectrophotometrically.

Results

All the three strains namely *Chlorella*, *Chlorococcum* sp. and *Desmococcus* sp could grow well in modified media composition that is in higher concentrations of sodium bicarbonate. The results are shown in Figure 1.

Figure 1:Growth Rate (divisons/day) of *Chlorella vulgaris*, *Chlorococcum humicola* and *Desmococcus olivaceous* in different molar concentrations of Sodium bicarbonate

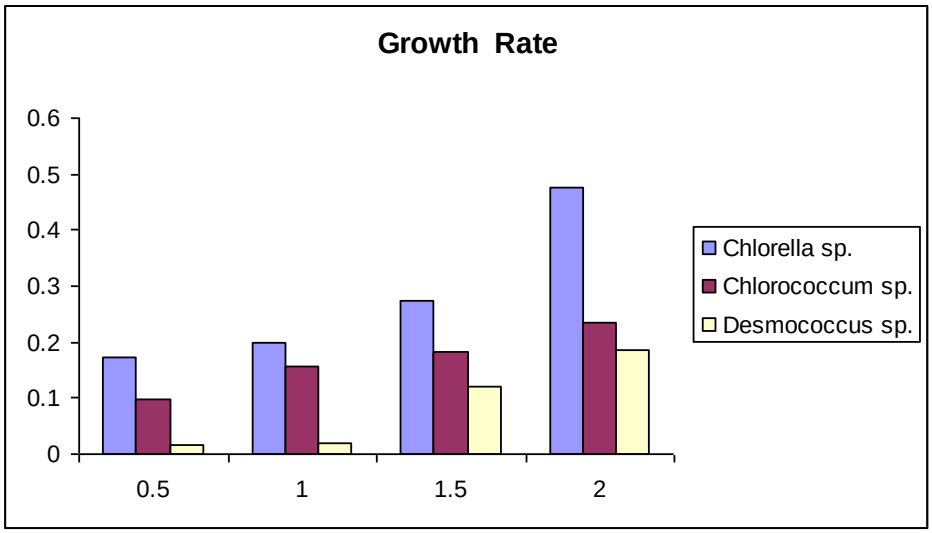


Figure 2: Chlorophyll a levels (µg/10^6 cells) in *Chlorella* sp., *Chlorococcum* sp. and *Desmococcus* sp., grown in different concentrations of Sodium bicarbonate

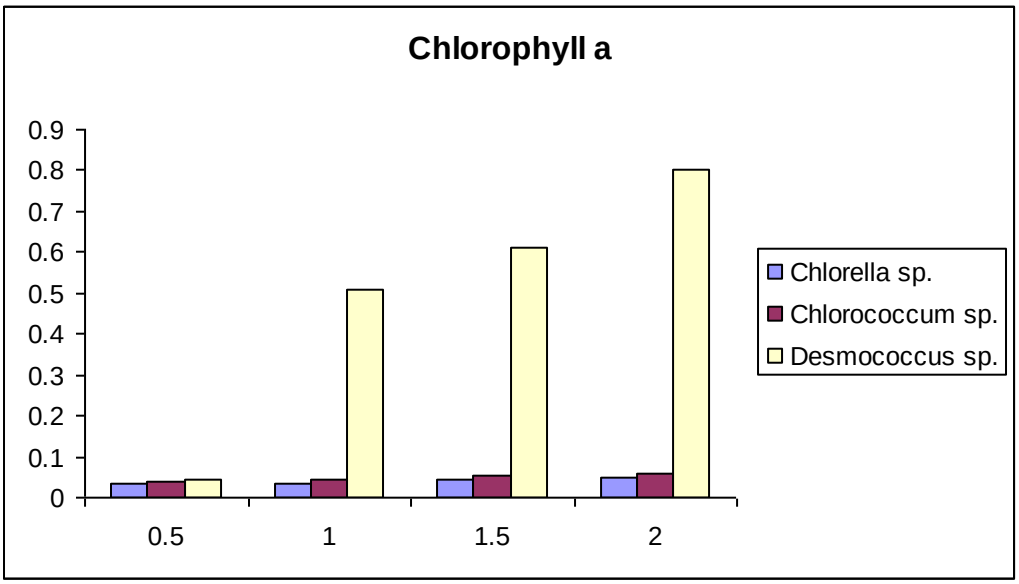


Figure 3: Chlorophyll b levels (µg/10^6 cells) of *Chlorella* sp., *Chlorococcum* sp. and *Desmococcus* sp., grown in different molar concentrations of Sodium bicarbonate

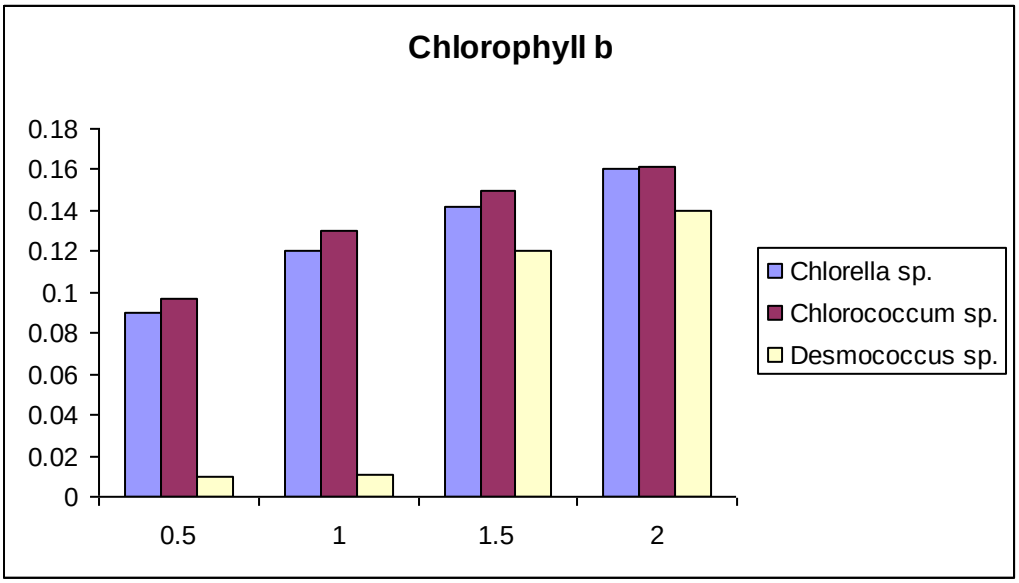


Figure 4: β-Carotene levels (µg/10^6 cells) of *Chlorella* sp., *Chlorococcum* sp. &*Desmococcus* sp., grown in different molar concentrations of Sodium bicarbonate

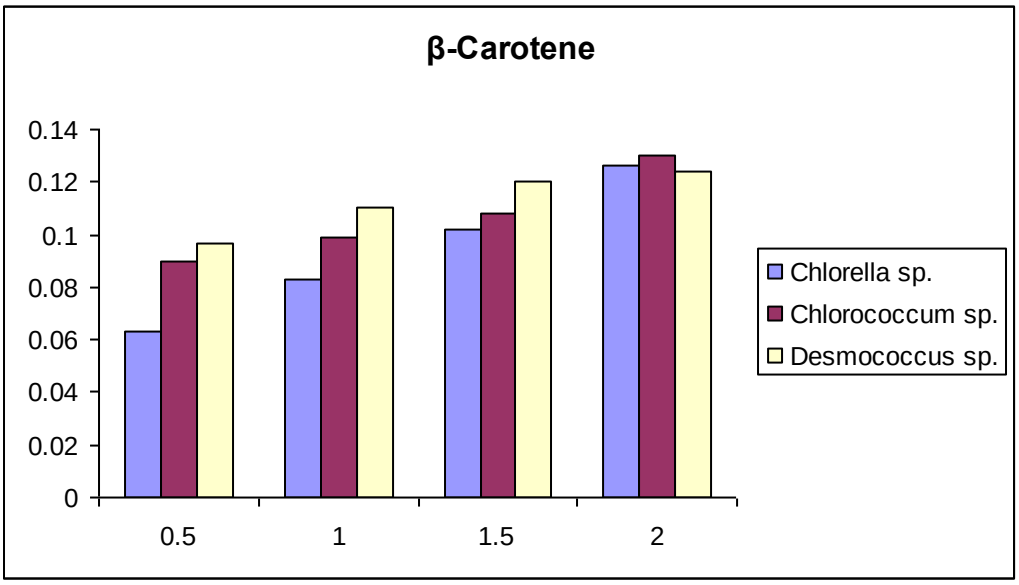


Fig. 5: Carbohydrate content (µg/10^6 cells) of *Chlorella* sp., *Chlorococcum* sp. *Desmococcus* sp., grown in different molar concentrations of Sodium bicarbonate

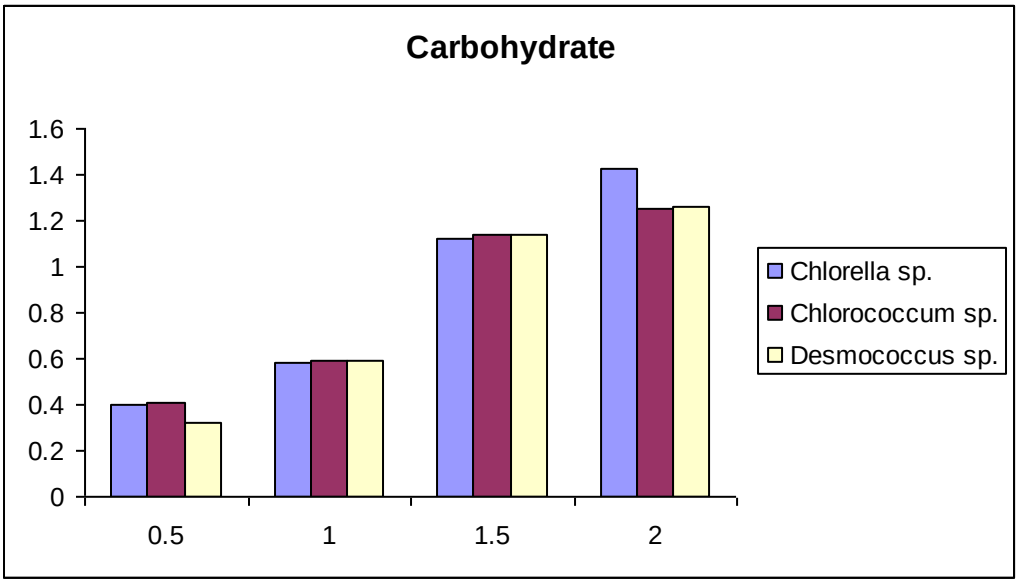


Fig. 6: Protein content (µg/10^6 cells) of *Chlorella* sp., *Chlorococcum* sp. and *Desmococcus* sp., grown in different molar concentrations of Sodium bicarbonate

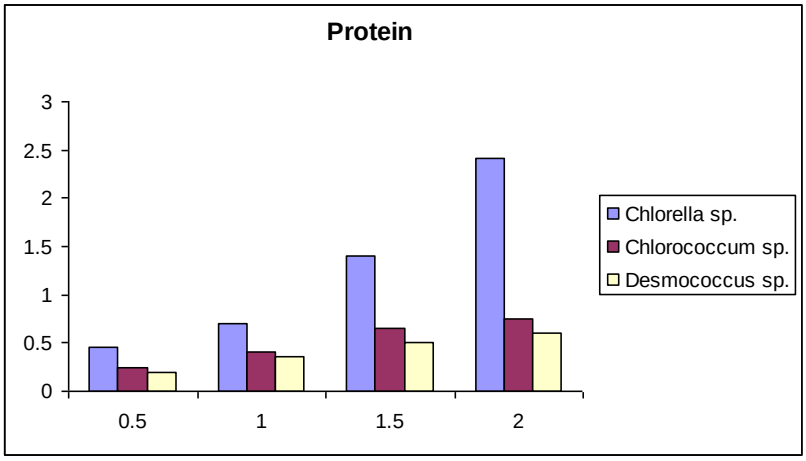
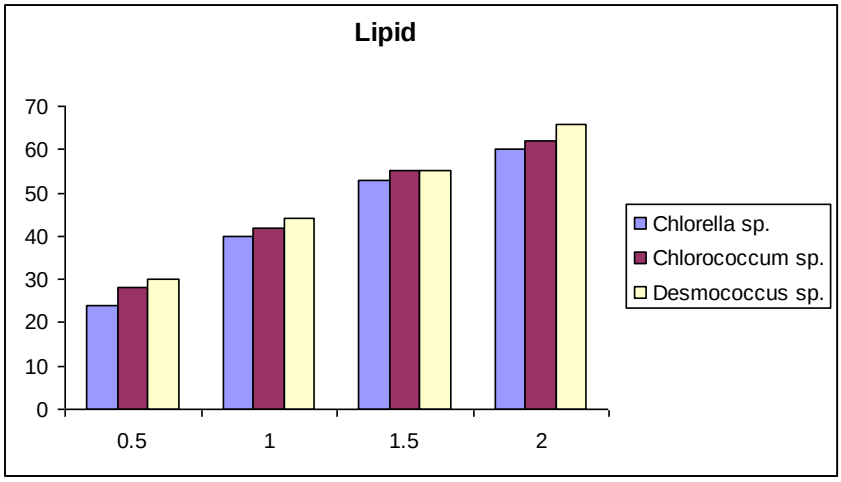


Fig.7: Lipid content (µg/10^6 cells) of *Chlorella* sp., *Chlorococcum* sp. and *Desmococcus* sp., grown in different molar concentrations of Sodium bicarbonate



Generally there was an increase in growth with increasing bicarbonate levels in all the micro algae. The maximum growth rate was recorded in *Chlorella* strain with values of 0.4770 at 2.0 molar concentrations as compared with *Chlorococcum* sp and *Desmococcus* sp (Figure 1). In order to develop an effective CO_2 mitigation technology, it is necessary to select an efficient and fast growing microalgae strain which has a good CO_2 fixing efficiency and promising valuable components, in such condition it would be suitable to employ chlorella sp for this study. Microalgae have several potential an advantage over terrestrial crops from which biodiesel is currently generated, including higher growth rates and productivity; reduced competition for arable land and associated water resources and effective nutrient utilisation, etc. (Hu *et al.* 2008; Li *et al*. 2008; Ahmad *et al.*, 2011).

Chlorophyll a and b were determined in *Desmococcus* sp , *Chlorococcum* sp and *Chlorella* sp. at various concentrations of bicarbonate (Figs 2 and 3) .The results showed that *Desmococcus* sp reached highest values among three microalgae at 2.0 molar concentration of bicarbonate . Chlorophyll is the pigment that allows plants (including algae) to use sunlight to convert simple molecules into organic compounds via the process of photosynthesis. Of the several kinds of chlorophyll, chlorophyll a is the predominant type found in green plants and algae. *Chlorococcum* sp showed higher β-carotene values compared to other two strains (Fig 4). The carbohydrates and protein contents were more in *Chlorella* when compared to *Desmococcus* sp and *Chlorococcum* sp (Figs 5 and 6). These macromolecules from microalgae have considerable biotechnological potential including producing valuable substances for the food additives, cosmetics, bio-fuel and pharmaceutical industries. *Chlorella* sp. is also a potential candidate for the production of biomass which is used in aquaculture for feeding, nutraceutical food additives and animal feed as it is rich in vitamins. Moreover, microalgal production of polyunsaturated fatty acids (PUFAs) for their nutritive value in aquaculture systems and for human health applications has been recognized (Berge and Barnathan *et al.,* 2005; Khozin-Goldberg *et al.*,2011).

Among value added bio-chemicals, the best results were obtained in lipids contents with respect to the strains of *Chlorella*, *Chlorococcum* sp and *Desmococcus* sp. Among the three microalgae , *Desmococcus* sp produced enhanced a higher lipid content when compared with *Chlorococcum* sp and *Chlorella sp* but difference among the three microalgae was very less, with values ranging from 60, 62, 66 at 2.0 molar concentration of bicarbonate (Figure 7). From the above study, it was found that the lipid content of all the strains increased when they were grown in media supplemented with bicarbonate. A feasible microalgal CO_2 - mitigation model can effectively fix CO_2 and also convert biomass to different valuable byproducts (Ono and Cuello, 2006). Recent studies showed. The merit of cultivation of microalgae, CO_2 mitigation and bio-fuel production which could be combined in an economically sustainable manner, the feasibility of this strategy could be further enhanced by fixing CO_2 from industrial exhaust gases such as flue gases. CO_2 concentration plays an important role in the increase of lipid productivity (Wang *et al.,* 2008). Enzyme carbonic anhydrase is associated with the process of reversible hydration of carbon dioxide helping to increase the efficiency of photosynthesis in micro algae (Suzuki *et al.,* 1994).In recent years, focus has turned to the production of microalgae cellular storage lipids (namely triglycerides or TAGs) for the production of biodiesel as sustainable alternatives to petroleum fossil fuels (Chisti *et al.,* 2007; Mata *et al*.2010). It was found that at higher CO_2 concentrations, algae exhibited higher lipid productivity (Widjaja *et al.,* 2009). The present study reveals that bicarbonate is an effective carbon source for microalgal growth, as optimum bicarbonate concentration is beneficial for highest biomass production. The findings also indicate that CO_2 supply can strongly affect the microalgae growth and quick accumulation of biomass in all the tested three strains ultimately leading to higher lipid production. *C. vulgaris* strain exhibited fastest growth rate when tested (Jeong *et al.,* 2003).

Conclusions

In summary, the findings suggest that bicarbonate addition can significantly increase photosynthetic efficiency and production of cellular compounds including pigments and lipids in microalgae, although the responses are species specific when compared under similar conditions. These findings also indicate that higher yields of valuable lipid and pigment compounds may be promoted by utilising bicarbonate addition in commercial production systems *Chlorella vulgaris* can be employed in large scale systems for flue gas mitigation as it has exhibited higher tolerance to increased bicarbonate levels.

Acknowledgements

We thank the Secretary and Principal, R.K.M. Vivekananda college, Chennai 600 004, India, for providing us with the necessary infrastructure and facilities required for the study.

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