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Studies on Growth and Biochemical Analysis of Three Microalgal Strains on Different Molar Concentration of Sodium Bicarbonate

KEYWORDS: Sodium bicarbonate ($NaHCO_3$), Microalgae, Biochemical

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ABSTRACT *In this present investigation an attempt was made to study the Bicarbonate tolerance of selected micro algal strains (Scenedesmus sp. Botryococcus sp. and Euglena sp.). The three microalgal strains were collected from the Presidency College, Chennai, India. These strains were grown in different molar concentrations of $NaHCO_3$ in two different medium BBM and BG11 without any changes in other substances of media. The Optical density (OD) was measured at regular interval time of 24hrs at 680nm employing UV-Vis Spectrophotometer. Growth rate and Biochemical composition were investigated in this study. All the three microalgal strains were grown well in the 0.1 molar concentration of $NaHCO_3$ in both BBM and BG11 media but the best growth rate was showed in BG11 media when compared to BBM. The highest growth rate was seen in Euglena sp. and lipid content was found high in Botryococcus sp. at 0.1 molar concentration of $NaHCO_3$.*

Introduction

Microalgae are aquatic unicellular organisms in both fresh and marine water having unique potential to synthesize valuable natural products (Plaza *et al.,* 2009; Plaza *et al.*, 2010). In recent years, lipid storage in microalgal cells turns the focus on biodiesel production, Triglycerides present in storage lipid used as a source for biodiesel and as a sustainable alternative for petroleum fuels (Mata *et al,*. 2010). Microalgae promises to be a suitable and efficient energy resource that can convert atmospheric carbon dioxide into biomass, fatty acid and lipids. Microalgae are low cost effective to culture, characterized by rapid growth and high biomass production, which utilizes bicarbonate as the external source of carbon for photosynthesis (Munoz and Beer, 1994). Few algae are capable of uptake of carbon dioxide directly (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 (Badger *et al.,* 1980). Microalgae can fix CO_2 from different sources which can be categorized as CO_2 from atmosphere, CO_2 from industrial flue gases, and fixed CO_2 in the form of soluble carbonates ($NaHCO_3$/Na_2CO_3). Carbon dioxide (CO_2) could be directly fed into microalgae culture for biofixation but sometimes it is difficult to obtain a stable and consistent supply of CO_2 unless the location of microalgae cultivation system is very close to a factory or a power plant. The major objective of this study was to optimize the growth rate of three different microalgal strains in different molar concentrations of $NaHCO_3$ to compare BBM and BG11 media, and analysis of biochemical composition from obtained microalgal biomass.

Materials and Methods

Three green microalgae, *Scenedesmus* sp., *Botryococcus* sp., and *Euglena* sp., were selected for this experiments. The strains were obtained from Culture Collection of Presidency College, Chennai, India. The aforementioned strains were explored in terms of Growth and biochemical study in different molar concentration of $NaHCO_3$ in two different medium (BBM and BG11). BBM and BG11 culture media were selected and prepared for the growth of microalgae strains, in which BG11 consists of carbonate source in it. Then different molar concentrations (0.1, 0.5, 1.0, 1.5, and 2.0) of $NaHCO_3$ salt was weighed and allowed to dissolve in BBM and BG11 media for carbon source and Control were prepared having normal BBM and BG11 without any strain inoculation. The pH was adjusted to 7.5 in each of the flasks using pH tutor meter (Eutech instruments).

Light condition

For our analysis, white inflorescence lamps (Crompton 40W, Cool day light 6500 K) at an intensity of 2000 lux in a 12:12 light and dark regime were employed and the temperature was adjusted to 25°C for all the flasks.

Analytical method

The Optical Density of the three microalgal cultures *Scenedesmus* sp. *Botryococcus* sp. and *Euglena* sp. were measured at regular interval time of 24 hrs by absorbance at 680 nm using spectrophotometer (Hitachi U-2900) till 15th day. At the end of the experiment, all the culture flasks were centrifuged and filtered. The pellet obtained biomass were oven dried, weighed and stored for further biochemical analysis.

Biochemical composition and analysis on microalgal biomass

Total Protein, Carbohydrate and Lipid was analyzed from biomass, based on Lowry *et al.,* 1951, Dubois *et al.,* 1956 and Ronald, 2001 methods respectively.

Results

Effect of Bicarbonate tolerance and utilization

Three microalgae strains *Scenedesmus* sp. *Botryococcus* sp. and *Euglena* sp. were investigated against different molar concentrations of $NaHCO_3$ in their media. Primary and suitable media for microalgae BG11 and BBM were selected and analyzed for this study. The three microalgal strains *Scenedesmus* sp. *Botryococcus* sp. and *Euglena* sp. showed simultaneous growth initially on both the media, but the rate was deviated after further growth based on the utilization and tolerance of sodium bicarbonate on each organism. The biomass and growth rate was higher at the 12th and 13th day after inoculation. *Scenedesmus* sp. showed slightly deviated growth rate in both the media but the growth rate was higher at 0.1 M concentration of $NaHCO_3$ in both BBM and BG11 media when compared to other strains. *Botryococcus* sp. gained growth rate at the same molar concentration as in

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Scenedesmus sp. but the rate was deviated between BBM and BG11 hence, BG11 showed the best growth rate when compared to BBM. *Euglena* sp. shows the highest growth rate when compared to both the microalgal strains. In overall analysis, 0.1 M concentration of bicarbonate and BG11 media was the feasible and suitable for uptake of carbonate and high growth biomass production of microalgal species. The growth rate of the three microalgal species in both BBM and BG11 are expressed in **Fig. 1 – 6**.

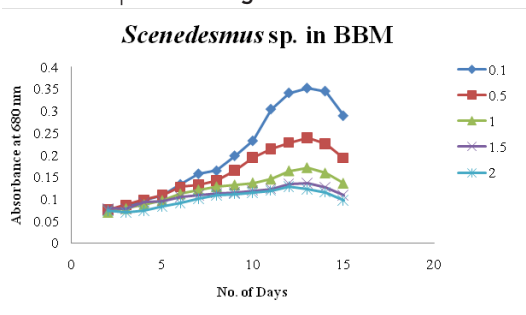

Fig. 1 Growth rate of *Scenedesmus* sp. in BBM under different levels of Sodium bicarbonate concentrations ($NaHCO_3$).

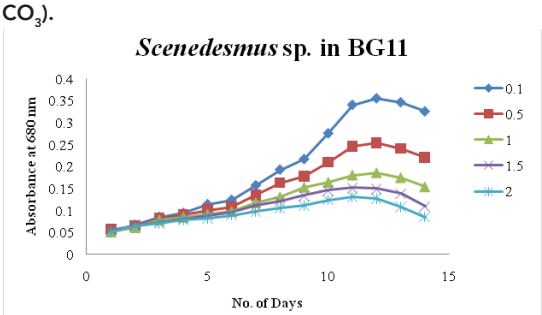

Fig. 2 Growth rate of *Scenedesmus* sp. in BG11 under different levels of Sodim bicarbonate concentrations ($NaHCO_3$).

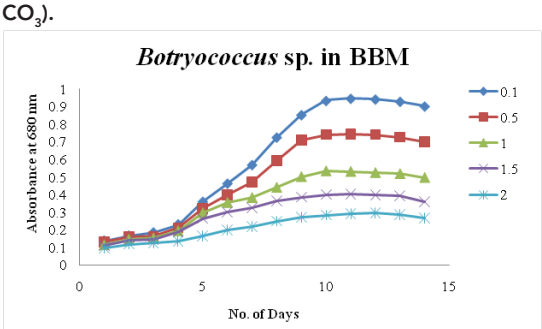

Fig. 3 Growth rate of *Botryococcus* sp. in BBM under different levels of Sodim bicarbonate concentrations ($NaHCO_3$).

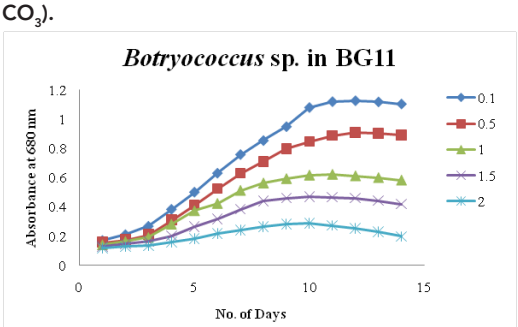

Fig. 4 Growth rate of *Botryococcus* sp. in BG11 under different levels of Sodim bicarbonate concentrations ($NaHCO_3$).

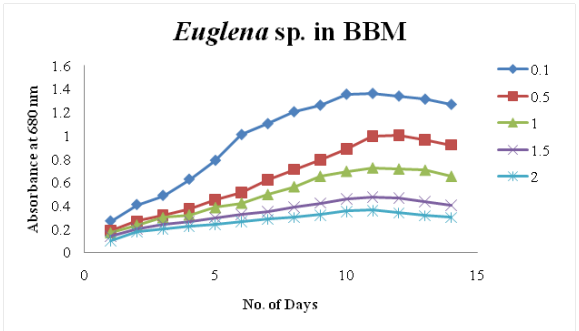

Fig. 5 Growth rate of *Euglena* sp. in BBM under different levels of Sodim bicarbonate concentrations ($NaHCO_3$).

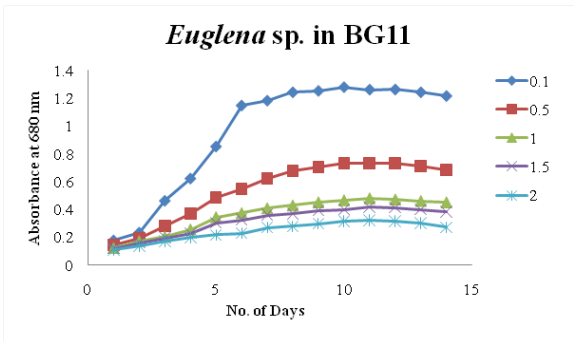

Fig. 6 Growth rate of *Euglena* sp. in BG11 under different levels of Sodim bicarbonate concentrations ($NaHCO_3$).

Total microalgal biomass

The total biomass obtained from three microalgal strains was **1.522 gl^{-1}, 1.684 gl^{-1}** and **1.886 gl^{-1}**, from *Scenedesmus* sp., *Botryococcus* sp. and *Euglena* sp. respectively. In which, *Euglena* sp. showed high biomass than the other two micro algal strains.

Biochemical composition

Total Protein

The total protein content of the three microalgal strains was estimated from the obtained algal biomass and expressed in mg g^{-1}. *Euglena* sp. gained 17.93 mg g^{-1}; *Botryococcus* sp. showed 14.57 mg g^{-1} and *Scenedesmus* sp. obtained 18.21 mg g^{-1}. *Scenedesmus* sp. secured the highest protein among the three microalgal strains **(Fig.7)**.

Total Carbohydrate

The total carbohydrate was estimated from the biomass, *Scenedesmus* sp. obtained high amount of carbohydrate when compared to others. *Scenedesmus* sp. 36.74 mg g^{-1}, *Botryococcus* sp. 31.42 mg g^{-1} and *Euglena* sp. 33.24 mg g^{-1} of carbohydrate present in it **(Fig.7)**, follows less than *Scenedesmus* sp.

Total Lipid

As we expect, the total lipid content of *Botryococcus* sp. was the highest when compared to *Scenedesmus* sp. and *Euglena* sp. 50.62 mg g^{-1}, 41.24 mg g^{-1} and 45.5 mg g^{-1} respectively. The overall biochemical composition Carbohydrate contributed 45%, Protein 33% and Lipid 17% in terms of percentage from the three microalgal strains **(Fig.7)**.

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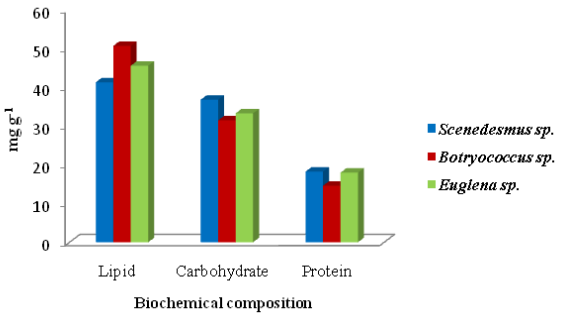

Fig. 7 Biochemical composition of micro algal biomass

Discussion

The microalgae have their own distinct applications in the field of algal biotechnology. *Botryococcus* a green microalga considered as a potent resource for fuel due its ability on high production of hydrocarbons. *Botryococcus* also involved in extraction of various carotenoids form it. Free fatty acids such as C_{18} and C_{16} fatty acids were isolated and studied form *Botryococcus braunii* and showed high amount of lipid content thus, may equip in third generation biofuel (Dragone *et al.*, 2010). The lipid content of all the microalgal strains increased when they were grown in media supplemented with bicarbonate salt, *Scenedesmus* strain showed highest accumulation of lipid along with highest growth response (Devgoswami *et al.*, 2011). *Scenedesmus* sp. could convert approximately 15-25% of atmospheric CO_2 into biodisel (Ho *et al.*, 2010), (Mandal and Mallick, 2009). From the present investigation, *Botryococcus* sp. found as the high lipid producer than the other strains as we expect, 50.62 mg of lipid form 1g of algal biomass. It did not show any increase in biomass when compared to other microalgal strains, but compared to different concentrations of $NaHCO_3$ 0.1 M was the best in high yielding of biomass. Freshwater *Scenedesmus obliquus* was one of best lipid producer in large scale level due to high lipid production (da silva *et al.*, 2008). Algal biomass will potentially increased by optimizing the culture medium, *Scenedesmus* sp. strain JPCC GA0024 showed high lipid content equivalent to highest lipid producers but not high than *Botryococcus braunii* (Matsunaga *et al.*, 2009). *Euglena* sp. accumulates somewhat high amount of lipid than *Scenedesmus* sp. and increase in its biomass than other two strains. Thus, *Euglena* sp. is viable in production of high biomass at 0.1 M concentrations of $NaHCO_3$. *Scenedesmus* sp. acquired less biomass than the other strains but produce high biomass at 0.1 M concentration by comparing different concentrations of $NaHCO_3$. Addition of sodium bicarbonate is a feasible strategy to control cellular abundance and concentrations of pigments and lipids.

Conclusion

An attempt was made to study the Bicarbonate tolerance by three different Micro algae viz., *Scenedesmus* sp, *Botryococcus* sp. and *Euglena* sp. at different concentration of $NaHCO_3$ dissolved in BBM and BG11 under *In-vitro* condition. At 0.1M concentration of $NaHCO_3$, the different micro algal strains *Scenedesmus* sp., *Botryococcus* sp. and *Euglena* sp. showed highest accumulation of lipid with highest growth rate. The technical data presented in this study are of relevance and value for further development and will generate additional interest in the field of algal technology. In future these algae can be grown in different concentrations of sodium bicarbonate ($NaHCO_3$) and the optimized concentration can be employed in biofuel production and Phycoremediation processes.

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