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 NaHCO₃ 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 24 hrs 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 NaHCO₃ 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 NaHCO₃.

**Materials and Methods**

Three green microalgal, *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 NaHCO₃ in two different medium (BBM and BG11). BBM and BG11 culture media were selected and prepared for the growth of microalgal 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 NaHCO₃ 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 microagal 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 microalgal strains *Scenedesmus* sp. *Botryococcus* sp. and *Euglena* sp. were investigated against different molar concentrations of NaHCO₃ in their media. Primary and suitable media for microalgal 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 NaHCO₃ in both BBM and BG11 media when compared to other strains. *Botryococcus* sp. gained growth rate at the same molar concentration as in...
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 (NaHCO₃).**

**Fig. 2 Growth rate of Scenedesmus sp. in BG11 under different levels of Sodium bicarbonate concentrations (NaHCO₃).**

**Fig. 3 Growth rate of Botryococcus sp. in BBM under different levels of Sodium bicarbonate concentrations (NaHCO₃).**

**Fig. 4 Growth rate of Botryococcus sp. in BG11 under different levels of Sodium bicarbonate concentrations (NaHCO₃).**

**Fig. 5 Growth rate of Euglena sp. in BBM under different levels of Sodium bicarbonate concentrations (NaHCO₃).**

**Fig. 6 Growth rate of Euglena sp. in BG11 under different levels of Sodium bicarbonate concentrations (NaHCO₃).**

**Total microalgal biomass**

The total biomass obtained from three microalgal strains was 1.522 g l⁻¹, 1.684 g l⁻¹ and 1.886 g l⁻¹, from Scenedesmus sp., Botryococcus sp. and Euglena sp. respectively. In which, Euglena sp. showed high biomass than the other two microalgal 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. 14.57 mg g⁻¹ and Scenedesmus sp. 18.21 mg g⁻¹. Scenedesmus sp. secured the highest protein among the three microalgal strains (Fig.7).

**Total Carbohydrate**

As we expect, the total lipid content of Botryococcus sp. was the highest 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).
Discussion

The microalgae have their own distinct applications in the field of algal biotechnology. Botryococcus, a green microalga considered as a potential resource for fuel due to its ability on high production of hydrocarbons. Botryococcus also involved in extraction of various carotenoids form it. Free fatty acids such as C16 and C18 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 (Devgovswaremi et al., 2011). Scenedesmus sp. could convert approximately 15-25% of atmospheric CO2 into biodiesel (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. 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 NaHCO3. Scenedesmus sp. acquired less biomass than the other strains but produce high biomass at 0.1 M concentration by comparing different concentrations of NaHCO3. 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 NaHCO3 dissolved in BBM and BG11 under In-vitro condition. At 0.1M concentration of NaHCO3, 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 (NaHCO3) and the optimized concentration can be employed in biofuel production and Phycoremediation processes.

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