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Biomass and Lipid Production Potential of Economically Important Marine Microalgal Strains

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A B S T R A C T

Four marine microalgal strains *viz*. *Pavlova* sp. ABT 102, *Chromulina* sp. ABT 103, *Thalassiosira* sp. AMS 5 and *Chlorella* sp. AMS 6 were tested for their biomass and lipid productivities through bench-scale studies to assess their potential for production of value added products. Among the four different marine microalgal strains *Pavlova* sp. ABT 102 isolate showed maximum volumetric biomass productivity of 0.048 g $L^{-1}d^{-1}$ followed by *Chlorella* sp. AMS 6 (0.035 g $L^{-1}d^{-1}$), *Thalassiosira* sp. AMS 5 (0.021 g $L^{-1}d^{-1}$) and *Chromulina* sp. ABT 103 (0.019 g $L^{-1}d^{-1}$). The highest volumetric lipid productivity was recorded by *Chromulina* sp. ABT 103 (11.12 mg $L^{-1}d^{-1}$) followed by *Pavlova* sp. ABT 102 (10.65 mg $L^{-1}d^{-1}$), *Thalassiosira* sp. AMS 5 (8.77 mg $L^{-1}d^{-1}$) and *Chlorella* sp. AMS 6 (7.73 mg $L^{-1}d^{-1}$). Among four algal strains, *Chlorella* sp. AMS 6 could effectively utilise sodium bicarbonate as an external inorganic carbon source in the growth medium. It was found that the addition of inorganic carbon source (sodium bicarbonate) in the algal strain AMS 6 *Chlorella* sp. enhanced biomass and lipid productivity by 6.5 and 7.6%, respectively. This strain when grown in 1-m^2 open raceway pond with and without CO_2 supplementation showed volumetric and areal biomass productivities of 0.046 g L^{-1} d^{-1} and 6.9 g m^{-2} d^{-1} in CO_2 supplemented treatment and 0.032 g L^{-1} d^{-1} and 4.8 g m^{-2} d^{-1} for control, respectively. Total lipid content of *Chlorella* sp. AMS 6 with and without CO_2 supplementation was 28-30% and did not show significant variation. The present study showed the potential of microalga *Chlorella* sp. AMS 6 to be utilised for biomass and lipid production for commercial applications.

Introduction

Microalgae are considered as potential biomass feedstock for the production of food, feed, fuels, nutraceuticals, cosmeceuticals, bioactive compounds and green chemicals (Slocombe and Benemann, 2016). Microalgae are important sources of commercially important high-value chemicals including carotenoids (Borowitzka, 2010), long-chain polyunsaturated fatty acids (Kyle, 1996; Ratledge, 2004) and phycobilins (Singh *et al.*, 2005). The great potential of marine microalgae for

applications, as such or as extracts, in areas so diverse as human nutrition and feed in aquaculture, as biofertilisers and in treatment of effluents, as anti-inflammatory, antiallergic and analgesic agents, among others, have been extensively reported (de Jesus Raposo *et al.,* 2013).

Many researchers have attempted to develop commercial products from microalgae as a source of lipids and carotenoids. Venkataraman *et al.,* advocated the use of microalgae as a source of single-cell protein (Venkataraman *et al.,* 1977). *Chlorella* and *Spirulina* arepopular health foods in Japan, Taiwan and Mexico and they were perhaps the first commercialised microalgal strains (Soong, 1980). This was followed in the 1980s by the commercialisation of β-carotene production from *Dunaliella salina* (Borowitzka, 2010; Borowitzka, 1989) astaxanthin from *Haematococcus pluvialis* in the1990s (Lorenz and Cysewski, 2000) and Docosahexaenoic acid DHA from *Crypthecodinium cohnii* (Kyle, 1996).

Microalgae constitute an important source of bioactive compounds used in a variety of nutraceutical and pharmaceutical applications. Among them, the omega-3 long-chain polyunsaturated fatty acids (*n*-3 LC-PUFA), such as Eicosapentaenoic acid (EPA, 20:5 *n*-3), and Docosahexaenoic (DHA, 22:6 *n*-3) acid, are known for their beneficial effects on human and animal health. As of today, *n*-3 LC-PUFAs are mainly obtained from marine fish oils. While microalgae synthesize *n*-3 LC-PUFA, fish usually obtain EPA from microalgae *via* bioaccumulation through the food chain, which increases the susceptibility to contamination of fish-derived omega3 and 6 fatty acids by pollutants such as heavy metals. In view of growing awareness about environment and health, the market for pure vegetarian source of food, feed and biomaterials is showing exponential growth in recent times. Apart from plants, algae are potential vegetarian and eco-friendly sources of high value lipids, proteins and carbohydrates. In addition to nutrients, marine algal strains are also rich in various valuable bioactive compounds[6]. Considering the above the present investigation was aimed at identifying and selecting potential marine microalgal strains for biomass and lipid production based on bench-scale and open raceway pond growth studies.

Materials and Methods

Microalgae strains and media

Marine microalgal strains of *Pavlova* sp. ABT 102, *Chromulina* sp. ABT 103, *Thalassiosira* sp. AMS 5 and *Chlorella* sp. AMS 6 (Fig 1) were obtained from algal germplasm collections facility of Biotechnology division of Aban Group in Chennai, Tamil Nadu, India. Cultures were maintained in the standard growth media and growth studies were carried out for *Pavlova* sp. and *Chromulina* sp. in F/2 medium (without silicate), under laboratory conditions. The F/2 medium[11]composition (mg L^{-1}) without silicate was as follows -$NaNO_3$- 75; $NaH_2PO_4 \cdot H_2O$- 5; Fe EDTA- 5; Thiamine – 0.1; Vitamin B_{12} – 0.5; Biotin – 0.1; $ZnSO_4 \cdot 7H_2O$ - 0.022; $MnCl_2 \cdot 4H_2O$ - 0.180; $CuSO_4 \cdot 5H_2O$ - 0.0098; $CoCl_2.6H_2O$ - 0.010; $Na_2MoO_4 \cdot 2H_2O$ - 0.006; Seawater – 1L.

Growth studies on *Thalassiosira* sp. was conducted in F/2 medium with the same composition mentioned above along with silicate ($Na_2SiO_3 \cdot 9H_2O$ -30 mg/L) supplementation. Growth studies on *Chlorella* sp. were carried out in BG-11 medium prepared with seawater. The medium composition (gL^{-1}) as follows: $NaNO_3$ -1.5; $CaCl_2.2H_2O$ – 0.036; Ferric ammonium citrate – 0.012; $Na_2EDTA.2H_2O$ – 0.001; K_2HPO_4 – 0.04; $MgSO_4.7H_2O$ – 0.075; Na_2CO_3 -0.02; Trace metal solution 1 ml L^{-1} [H_3BO_3 – 2.86 g L^{-1}; $MnCl_2.4H_2O$ – 1.81 g L^{-1}; $ZnSO_4.7H_2O$ – 0.222 g L^{-1}; $Na_2MoO_4.2H_2O$ – 0.39 g L^{-1}; $CuSO_4.5H_2O$ – 0.079 g L^{-1}; $Co(NO_3)_2.6H_2O$ – 0.049 g L^{-1}].

Propagation of seed inoculum

Pavlova sp., *Chromulina* sp., *Thalassiosira* sp. and *Chlorella* sp., were grown in 2 L flasks followed by 20L carboys with 10L culture medium, in the culture

room. From the carboys, 5L of microalgal cultures were transferred to 45L of respective culture medium specific to each algal strain i.e. F/2 medium with silicate, F/2 medium without silicate and BG 11 medium, sterilized with hypo (sodium hypochlorite) and neutralized with sodium thiosulphate in 60L capacity photobioreactors (PBR). The cultures were incubated at 25±1℃ at 30µE m^{-2} s^{-1} light intensity with 12/12 light/dark cycle under laboratory conditions.

Bench-scale growth studies

Experiments were carried out in 1000 mL Erlenmeyer flasks containing 540 mL of F/2 medium with silicate, F/2 medium without silicate and BG 11 medium, inoculated with 60 mL of optimally grown cultures of *Pavlova* sp., *Chromulina* sp., *Thalassiosira* sp., and *Chlorella* sp. The cultures were incubated at 25±1°C at 30µE m^{-2} s^{-1} light intensity with 12/12 light/dark cycle under laboratory conditions. To assess the impact of addition of the inorganic carbon source i.e. sodium bicarbonate, another study was carried out in 1000 mL Erlenmeyer flasks containing 540 mL of F/2 medium supplemented with and without 0.5 g/L of $NaHCO_3$ for *Pavlova* sp., and *Chromulina* sp. (without silicate), *Thalassiosira* sp. (with silicate) and BG-11 media for *Chlorella* sp., for a period of 10 days. At every two day intervals, 20 mL of sample was drawn from the culture and the following parameters were recorded: pH, TDS, Salinity, Conductivity, Absorbance (OD) and biomass dry weight

Dry weight measurement

Biomass estimation was carried out using a moisture analyser (Mettler Toledo HR-83P, LLC 1900, USA) with an inbuilt halogen lamp and an analytical balance (Ratha *et al.,* 2016). Heating occurs uniformly at a set temperature by a halogen lamp until sample reaches zero moisture. The final weight and moisture content were displayed after complete drying of the sample. The temperature range that can be set in this model of moisture meter was from 40 to 200°C. The balance resolution and repeatability were 0.0001 and ±0.0001 g, respectively, and the maximum weighing capacity of the balance was 50 g.

Whatman GF/C filters with sample codes were kept inside the aluminum pan of the moisture analyser and dried. Combined weights of aluminum pan with the filter papers were recorded. Filtration was carried out in a Tarson's membrane filtration assembly by vacuum suction. For marine algal strains, biomass was washed with distilled water followed by 0.65M ammonium formate solution and again with distilled water to remove excess salts. The filter containing the wet algal biomass was placed in the aluminum pan of the moisture analyser and dried. Dry weight of the algal sample was determined by subtracting the empty weight of the filter paper from the dry weight of filter paper with algal biomass. All dry weight measurements were carried out in triplicate, and the values were presented as mean ±SD.

Biochemical analyses

Total lipids in the alga were extracted and estimated according to the method of Folch *et al.,*. Protein was estimated using Lowry's method (Lowry *et al.*, 1951). Total carbohydrates were measured by the phenol-sulphuric acid method developed by Dubois *et al.,*. The total chlorophyll content was estimated following the method of McKinney[16]. Carotenoids were extracted with 85% acetone as described by Jensen, 1978.

Outdoor studies in 1-m^2Fibre reinforced plastic (FRP) raceway ponds

Outdoor studies were conducted in 1m^2 raceway pond for the cultivation of *Chlorella* sp. AMS 6.Experiments were carried out in 150L FRP ponds containing 135 L of modified BG 11 medium prepared with filtered seawater and inoculated with 15L of optimally grown cultures of *Chlorella* sp. AMS6 (Table 3). The ponds were initially washed with diluted commercial HCl (40% diluted). After

acid wash the ponds were rinsed with tap water 3 to 4 times. Algal cultures were grown in sterile nutrient-enriched medium in the laboratory for mass cultivation experiments. In order to complete the process of sterilization in mass cultivation experiments, the medium was chemically sterilized with sodium hypochlorite (20 l culture media – 50ppm chlorine concentration) for a period of 12 hours. After this, the residual free-chlorine was neutralized with sodium thiosulphate (2.85 mg per 1 mg of chlorine) for one hour. After neutralization process, the media was analysed for residual chlorine with a Hach spectrophotometer. The growth medium before algae inoculation is thus ensured to be free from chlorine residues.

For CO_2 supplementation studies, CO_2 from 40 kg cylinder was bubbled at a flow rate of 2 L per min using a gas rotameter using spargers installed at the bottom of one of the FRP raceway ponds. pH of the culture in the open race way pond supplemented with CO_2 was maintained between 8 and 8.5 during the study period to avoid gaseous CO_2 loss as the dissolved CO_2 will be retained in the culture in bicarbonate form between pH 8-8.5.

A solenoid valve system (Milwaukee SM100) was attached to the cylinder, which aided in the automatic cut-off of CO_2 supply, when the pH of the culture exceeded the set value. Daily evaporation loss was made up with filtered tap water. Light intensity and temperature were recorded thrice a day. Physicochemical parameters, pH, salinity, TDS, conductivity and absorbance were recorded daily. The growth rate was tracked by taking OD and dry weight measurements at regular intervals.

Results and Discussion

Growth studies on marine microalgal strains

Changes in physicochemical parameters of growth medium during the growth of algae

Microalgae isolates such as *Pavlova* sp. ABT102, *Chromulina* sp. ABT103, *Thalassiosira* sp. AMS 5 were grown in F/2, while *Chlorella* sp. AMS6 was cultivated in BG11 medium. The physicochemical changes observed in the growth media were monitored.

The initial pH in *Pavlova* sp. ABT102, *Chromulina* sp. ABT103, *Thalassiosira* sp. AMS5 and *Chlorella* sp. AMS6 gradually increased from 7.34, 7.85, 7.71 and 8.13 to 8.66, 8.18, 8.01 and 8.23, respectively at the end of the study period. The values of TDS, salinity and conductivity showed slight variations during the study period in all the treatments (Fig 2).

Growth kinetics

Growth performance of the four microalgae isolates - *Pavlova* sp. ABT102, *Chromulina* sp. ABT103, *Thalassiosira* sp, AMS5 grown in F/2 medium and *Chlorella* sp. AMS6 in BG11 medium were studied and their growth performance in terms of dry weight and optical density were monitored for 10 days.

Absorbance

Among the four different strains, *Pavlova*sp. ABT 102 exhibited highest growth with 0.633 absorbance at 750nm followed by *Chlorella* sp. AMS 6 (0.476), *Chromulina* sp. ABT 103 (0.413) and *Thalassiosira* sp. AMS5 (0.397) on 10^{th} Day (Fig3A).

Dry weight

Among the four different marine microalgae *Pavlova* sp. ABT102 isolate showed a maximum biomass concentration of 0.59 g L^{-1} on 10^{th} day of cultivation followed by *Chlorella* sp. AMS 6 of 0.44g L^{-1}, *Thalassiosira* sp. AMS 5 of 0.24 g L^{-1} and *Chromulina* sp. ABT 103 of 0.23 g L^{-1} on 10^{th} day, respectively (Fig 3B).

Pavlova sp. ABT 102 recorded highest volumetric productivity of 0.048 g $L^{-1}d^{-1}$ followed by *Chlorella* sp. AMS6 which recorded 0.035 g $L^{-1}d^{-1}$; whereas *Thalassiosira* sp. AMS5 and *Chromulina* sp. ABT103 recorded volumetric biomass productivities of 0.021 and 0.019g $L^{-1}d^{-1}$, respectively (Table 1).

Veronesia *et al.,* 2015 reported that the volumetric biomass productivity value for *P.lutheri* in F/2 medium as 0.015 g $L^{-1}d^{-1}$ which was 69% lower than the volumetric biomass productivity recorded in this experiment. Shah *et al.,* 2014 observed a volumetric biomass productivity of 0.028 g $L^{-1}d^{-1}$ for *P. lutheri* which was 42% less than the biomass productivity observed in the present study. Roleda *et al.,* 2013 reported volumetric biomass productivities of 0.290 g $L^{-1}d^{-1}$ and 0.227 g $L^{-1}d^{-1}$ for *Thalassiosira pseudonana* and *Chromulinao chromonoides*, cultivated in F/2 medium at 20°C, respectively. However, these values are very high and not at all comparable with the volumetric biomass productivity values reported for these strains in the literature. Moazami *et al.,* 2011 recorded a volumetric biomass productivity of 0.013 g $L^{-1}d^{-1}$ in *Chlorella* sp. PTCC6002. This value was 63% less than the productivity value observed for the *Chlorella* strain used in the present study.

Biochemical composition

Chlorophylls are the vital cellular pigments for microalgae growth measurement in culture systems. The volumetric chlorophyll productivity observed in the present study was as follows: *Chlorella* sp. AMS6>*Pavlova* sp. ABT102> *Chromulina*sp. ABT 103 > *Thalassiosira*sp. AMS5 (Table 2).

Among all the strains *Chromulina* sp. ABT 103 recorded highest lipid content of 59% followed by *Thalassiosira* sp. AMS5 (42%), *Pavlova* sp. ABT 102 (22.18%) and *Chlorella* sp. AMS 6 (22.09%). Interestingly the lipid content of the strains such as *Pavlova*sp. and *Chlorella* sp. with highest volumetric biomass productivities was only 22% when compared to the strains with low volumetric biomass productivities such as *Thalassiosira* sp. AMS5 and *Chromulina* sp. ABT 103 which recorded lipid contents of 42 and 59%, respectively (Table 2).

When comparing volumetric lipid productivities, *Chromulina* sp. ABT 103 recorded highest productivity of 11.12 mg/L/d followed by *Pavlova* sp. ABT 102, *Thalassiosira* sp. AMS5 and *Chlorella* sp. AMS 6 with volumetric lipid productivity values of 10.65, 8.77 and 7.73 mg $L^{-1}d^{-1}$, respectively (Table 2).

Chromulina sp. ABT 103 recorded highest volumetric carbohydrate productivity of 7.74 mg $L^{-1}d^{-1}$ followed by *Pavlova* sp. ABT 102, *Thalassiosira* sp. AMS5 and *Chlorella* sp. AMS 6 with volumetric lipid productivity values of 6.77, 3.22 and 2.20 mg $L^{-1}d^{-1,}$ respectively (Table 2). Carbohydrates are essentially intermediary reserves in some algae, due to the fact that they are required when nitrogen becomes limited, in lipid biosynthesis (Sahay and Braganza, 2016). Carbohydrates tend to accumulate in the stationary phase.

Highest volumetric protein productivity was recorded for *Chlorella* sp. AMS 6 followed by *Pavlova* sp. ABT 102, *Thalassiosira* sp. AMS5 and *Chlorella* sp. AMS 6.

Effect of sodium bicarbonate on biomass and biochemical composition of microalgae

High concentration of bicarbonate ion and low concentration of carbon dioxide are normally found in seawater (Israel and Gonzalez, 1996). Microalgae utilize bicarbonate and CO_2 as the external source of carbon for photosynthesis (Dixon *et al.,* 1987; Raven, 1991). Most of the microalgal strains 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). Under carbon limitation, photosynthesis in microalgae is very similar to C4 type plants with much higher affinity to CO_2. Microalgae normally accumulate carbon intracellularly and the uptake is driven by energy coupled Ci transport system (Wang and Spalding, 2006). To increase the efficiency of photosynthesis in microalgae, the enzyme carbonic anhydrase is associated with the process of reversible hydration of carbon dioxide (Suzuki, 1994). Sodium bicarbonate as a carbon source can play an important role in improving the metabolic efficiency

and biochemical composition of microalgae. An investigation was conducted under laboratory conditions on the growth and biochemical composition of four strains of marine microalgae supplemented with inorganic carbon (0.5 gL^{-1}).

Pavlova sp. ABT102 grown in the medium without sodium bicarbonate showed an increase of 33, 22, 30 and 14% in biomass, total chlorophyll, protein and lipid contents on day 10, respectively when compared to the cells grown in the medium supplemented with sodium bicarbonate (Fig.4). *Chromulina* sp. ABT103 grown without sodium bicarbonate (control) recorded an increase of 23, 20, 28, 27 and 19% in biomass, total chlorophyll, carbohydrate, protein and lipid contents on day 10, respectively when compared to the treatment supplemented with sodium bicarbonate (Fig 4).

Thalassiosira sp. AMS5 recorded an increase of 6, 0.45, 5 and 10% in biomass, total chlorophyll, carbohydrate, protein and lipid values on day 10 when compared to the treatment supplemented with sodium bicarbonate (Fig 4).

Interestingly *Chlorella* sp. AMS6 grown in the treatment supplemented with sodium bicarbonate in contrast to other algae strains recorded 6, 11, 5, 8 and 8% increase in the biomass, total chlorophyll, carbohydrate, protein and lipid contents on the 10^{th} day when compared to the control (Fig 4).

From the above observations, it was clearly shown that among the four different marine microalgae, only *Chlorella* sp. AMS6 isolate could utilise sodium bicarbonate effectively from the growth medium.

Geider[29] reported that at low cell density, the addition of inorganic carbon will reduce the pH thereby reducing the growth in the initial phase of inoculation. Addition of bicarbonate could have influenced a change in the pH and lowered the pigment concentration in the cells of the following cultures viz. *Pavlova* sp. ABT102, *Chromulina* sp. ABT103 and *Thalassiosira* sp. AMS5 as the initial cell density was low. (Pesheva *et al.*, 1994) observed that the growth of the marine microalga *Chlorococcum littorale* was suppressed for the first 3-4 days after inoculation with medium supplemented with 40% carbon dioxide. However the cells recovered from the initial stress and exhibited logarithmic growth later. The initial stress caused by CO_2 addition would have suppressed PSII activity, Pronina and Borodin[31] also observed intracellular acidification due to increased concentration of CO_2.

Hence, addition of sodium bicarbonate alters the pH of the growth medium. The change in the optimum pH values for *Pavlova* sp. ABT102, *Chromulina* sp. ABT103 and *Thallassiosira* sp. AMS5 by the addition of sodium bicarbonate would have impacted the growth of these algae strains in the initial phase of growth which resulted in relatively less growth and biomass production when compared to the control.

Mass cultivation of *Chlorella* sp.AMS6 in open raceway ponds

The microalga *Chlorella* sp. AMS6 was grown in the modified BG11 medium prepared with filtered seawater with and without CO_2 supplementation.

The study was conducted inside a polyhouse harbouring two $1m^2$ FRP raceway ponds. For phototrophic algal growth, light and temperature are very crucial. Hence, the light intensity and temperature inside and outside the polyhouse (ambient) were monitored and recorded three times per day (Table 4 & 5).

Solar radiation

The minimum solar radiation received was 2.64 MJ m^{-2} d^{-1} inside the polyhouse and 5.88 MJ m^{-2} d^{-1}outside the polyhouse, respectively during the experiment period. The day average light intensity was found to be 13.60 MJ m^{-2} d^{-1}inside thepolyhouse and 20.58MJ m^{-2} d^{-1}outside the polyhouse. The maximum light intensity inside and outside the

polyhouse observed was 22.51 MJ m^{-2} $d^{-1}$and 31.9 MJ m^{-2} d^{-1}, respectively (Table 4).

Temperature

Temperature inside the polyhouse and outside the polyhouse (ambient) was recorded three times a day (Table 5). The maximum temperature inside the polyhouse was 35.5°C, whereas it was 37°C outside the polyhouse. Similarly the minimum temperature recorded was 31°C inside the polyhouse and it was 32°C outside the polyhouse, respectively during the experiment period. The day average temperature recorded inside the polyhouse was 33.27°C and it was 35°C outside the polyhouse.

Physicochemical parameters

The values of pH, salinity, TDS and conductivity recorded at different time intervals did not show any significant variation in both the ponds where the alga *Chlorella* sp. AMS6 was grown with and without CO_2 bubbling (Fig 5). In the raceway pond supplemented with CO_2, the pH of the culture was maintained between 8.0 to 8.5 whereas in the pond without CO_2supplementation, the initial pH of 8.15 rose to 8.24 at the end of growth period (Fig 5)

Growth dynamics - biomass productivity and photosynthetic efficiency

The 1 m^2 raceway pond containing 150 litres of modified BG11 medium with and without CO_2 supplementation revealed the following observations.

Fig 6a shows the increase in absorbance value from 0.088 to 0.845 for the pond supplemented with CO_2 and 0.593 for the control pond. The initial biomass of 0.04 g L^{-1} gradually rose to 0.36 g L^{-1} in control (without CO_2 supplementation) pond whereas the final biomass concentration reached 0.517 g L^{-1} in the pond supplemented with CO_2 (Fig 6b).

The biomass productivity was 43% higher in the treatment supplemented with CO_2than that of control. The alga grown in the pond supplemented with CO_2 showed maximum growth and biomass productivity (Fig 6, Table6). The volumetric and areal productivity in the ponds with CO_2 supplementation were 0.046 g L^{-1} d^{-1} and 6.9 g m^{-2} d^{-1}, respectively; whereas in the control it was 0.032 g L^{-1} d^{-1} and 4.8 g m^{-2} d^{-1}(Table 6).

Table.1 Volumetric biomass productivities of marine microalgae strains *Pavlova* sp. ABT 102, *Chromulina* sp. ABT 103, *Thalassiosira* sp. AMS 5 and *Chlorella* sp. AMS 6 under laboratory conditions

S.No.	Name of the strain	Biomass concentration (g L^{-1})	Volumetric biomass productivity (g L^{-1} d^{-1})
1	*Pavlova* sp. ABT102	0.590	0.048
2	*Chlorella* sp. AMS6	0.443	0.035
3	*Thalassiosira* sp. AMS5	0.240	0.021
4	*Chromulina* sp. ABT103	0.230	0.019

Table.2 Biochemical composition of selected algal strains under indoor conditions

Organism	Total Lipid (mgL^{-1})	% of lipids on the basis of dry wt	Volumetric lipid productivity (mgL^{-1} d^{-1})	Total Carbohydrates (mg L^{-1}	Volumetric carbohydrate productivity (mgL^{-1} d^{-1})	Total Chlorophyll (mg L^{-1})	Volumetric chlorophyll productivity (mg L^{-1} d^{-1})
***Pavlova* sp. ABT 102**	130.87±0.01	22.18	10.65	83.23 ±0.08	6.77	3.93 ±0.13	0.32
***Chlorella* sp. AMS 6**	97.84±0.18	22.09	7.73	27.81 ±0.09	2.2	5.18±0.01	0.41
***Thalassiosira* sp. AMS 5**	100.28±0.10	41.78	8.77	37.91 ±0.06	3.32	1.85±0.07	0.16
***Chromulina* sp. ABT 103**	134.67±0.12	58.55	11.12	93.64 ±0.03	7.74	3.25±0.01	0.27

Table.3 Dimensions of Fibre Reinforced Plastic (FRP) Raceway ponds

Parameters	Dimensions
Length inner (m)	2.22
Width inner (m)	0.50
Depth (m)	0.26
Partition wall length (m)	1.79
Partition wall width (m)	0.20
Bottom area (m2)	0.76
Volume of FRP raceway pond (L)	150

Table.4 Light intensity/solar radiation recorded during the experimental period both inside the Polyhouse and outside of Polyhouse.

Day	Light inside polyhouse (MJ m^{-2} d^{-1})			Light outside polyhouse (MJ m^{-2} d^{-1})		
Time	9.30 AM	12.30 PM	4.30 PM	9.30 AM	12.30 PM	4.30 PM
0	10.67	22.51	2.72	24.11	30.18	6.43
1	17.33	22.16	2.75	26.50	31.33	6.13
2	17.02	22.16	2.71	23.83	31.92	5.88
3	10.84	19.87	4.17	19.97	30.43	9.73
4	16.95	22.51	2.72	24.11	30.18	6.43
5	17.33	22.16	2.75	26.50	31.33	6.13
6	17.02	22.16	2.64	23.83	31.92	5.88
7	17.26	19.52	4.65	19.97	30.43	9.73
8	16.95	22.51	2.72	24.11	30.18	6.43
9	17.02	22.16	2.71	23.83	31.92	5.88
10	17.33	22.16	2.75	26.50	31.33	6.13
Average	15.97	21.81	3.03	23.93	31.01	6.80
	13.60			**20.58**		

Table.5 Temperature recorded during the experimental period both inside and outside of the polyhouse.

Day	Light inside polyhouse (MJ m^{-2} d^{-1})			Light outside polyhouse (MJ m^{-2} d^{-1})		
Time	9.30 AM	12.30 PM	4.30 PM	9.30 AM	12.30 PM	4.30 PM
0	32.50	33.00	32.50	33.50	34.00	33.00
1	33.50	35.50	33.00	35.00	37.00	35.00
2	33.00	35.00	32.00	34.00	36.00	35.50
3	31.00	34.50	32.50	32.00	35.00	34.50
4	33.00	32.00	33.00	35.00	34.00	35.00
5	34.00	33.00	34.50	36.00	35.00	36.00
6	33.00	35.00	33.50	35.00	37.00	35.00
7	32.50	33.00	34.00	35.00	36.00	35.00
8	33.00	32.00	33.00	35.00	34.00	35.00
9	33.00	35.00	32.00	34.00	36.00	35.50
10	34.00	33.00	34.50	36.00	35.00	36.00
Average	32.95	33.73	33.14	34.59	35.36	35.05
	33.27			**35.00**		

Table.6 Biomass productivity and changes in biochemical composition in *Chlorella* AMS 6 cultivated in 1 m^2 open raceway pond

Treatment	With CO_2	Without CO_2
Biomass		
Biomass concentration (g L^{-1})	0.85	0.59
Volumetric biomass productivity (g $L^{-1}d^{-1}$)	0.05	0.03
Areal biomass productivity (g m^{-2} d^{-1})	6.89	4.80
Specific growth rate (μ)	0.21	0.22
Generation time (days)	3.10	3.20
No. of divisions per day	0.32	0.32
Lipid		
Total Lipid (mg L^{-1})	149.50	109.27
% of lipids on the basis of dry wt	28.97	30.35
Volumetric lipid productivity (mg $L^{-1}d^{-1}$)	13.21	9.55
Areal lipid productivity (g m^{-2} d^{-1})	1.98	1.43
Carbohydrate		
Total Carbohydrate (mg L^{-1})	58.39	40.41
% of carbohydrate on the basis of dry wt	11.32	11.23
Volumetric carbohydrate productivity (mg $L^{-1}d^{-1}$)	5.17	3.48
Areal carbohydrate productivity (g m^{-2} d^{-1})	0.77	0.52
Protein		
Total Protein (mg L^{-1})	198.40	142.20
% of protein on the basis of dry wt	38.45	39.50
Volumetric protein productivity (mg $L^{-1}d^{-1}$)	17.60	12.45
Areal protein productivity (g m^{-2} d^{-1})	2.64	1.87
Chlorophyll		
Total Chlorophyll (mg L^{-1})	6.89	5.08
% of chl on the basis of dry wt	1.34	1.41
Volumetric chlorophyll productivity (mg $L^{-1}d^{-1}$)	0.61	0.45
Areal chlorophyll productivity (g m^{-2} d^{-1})	0.092	0.068

Table.7 Photosynthetic efficiency estimated for three different energy contents in the dry algal biomass

Photosynthetic efficiency (PE)	Areal biomass productivity (g m-2 d-1)		
	Biomass energy content (MJ m-2 d-1)		
100%	1251	1126	1023
10%	125	113	102
5%	62.5	56.3	51.2
4%	50.0	45.0	40.9
3%	37.5	33.8	30.7
2%	25.0	22.5	20.5
1%	12.5	11.3	10.2
0.9%	11.3	10.1	9.2
0.8%	10.0	9.0	8.2
0.7%	8.8	7.9	7.2
0.6%	7.6	6.9	6.2
0.5%	6.3	5.6	5.1
0.4%	5.0	4.5	4.1
0.3%	3.8	3.4	3.1

Assumption*: Average solar radiation - 22.51 MJ* m^{-2} d^{-1}

Fig.1 Photomicrographs of microalgae; [A] *Pavlova* sp. ABT102, [B] *Chromulina* sp. ABT103, [C] *Thalassiosera* sp. AMS5, [D] *Chlorella* sp.AMS 6. Scale bar 10 µm.

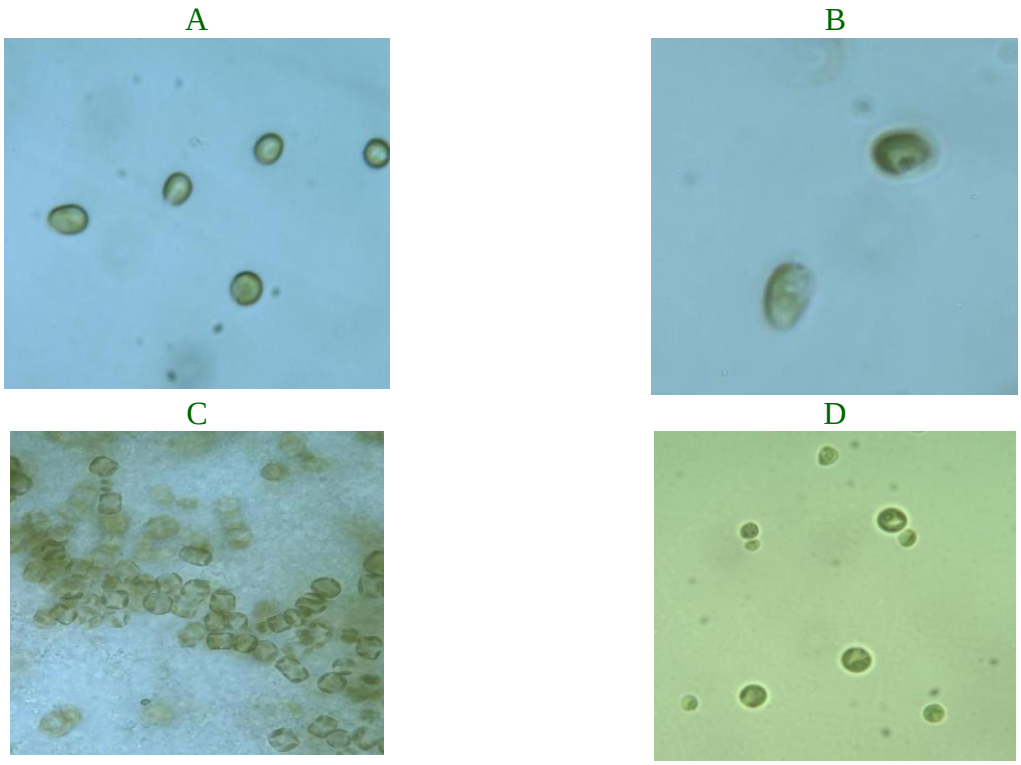


Fig .2 Physico-chemical changes in the growth medium of [a] *Pavlova* sp. ABT 102 [b] *Chromulina* sp. ABT103 [c] *Thalassiosira* sp. AMS 5 [d] *Chlorella* sp.AMS 6 under laboratory conditions.

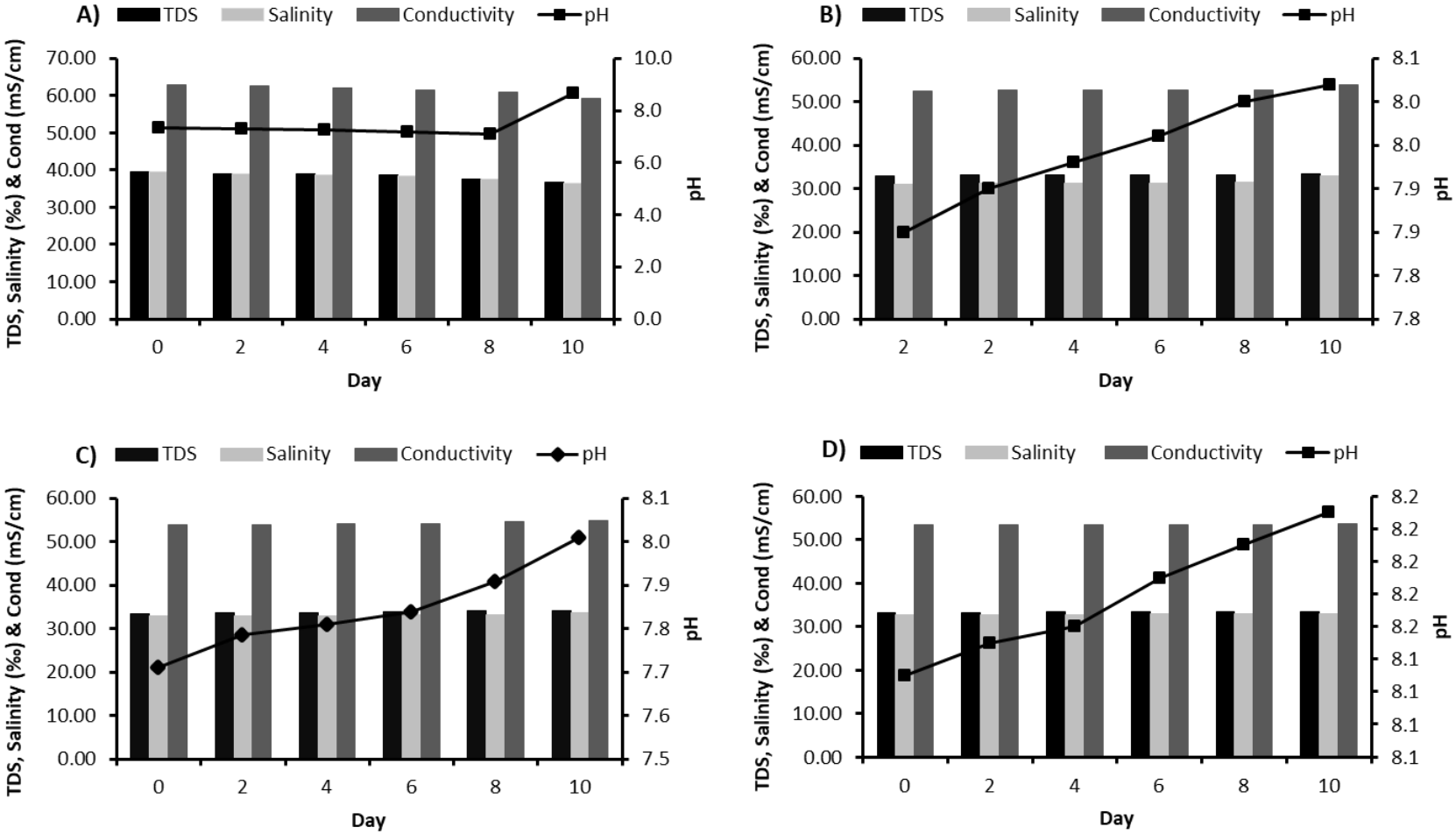
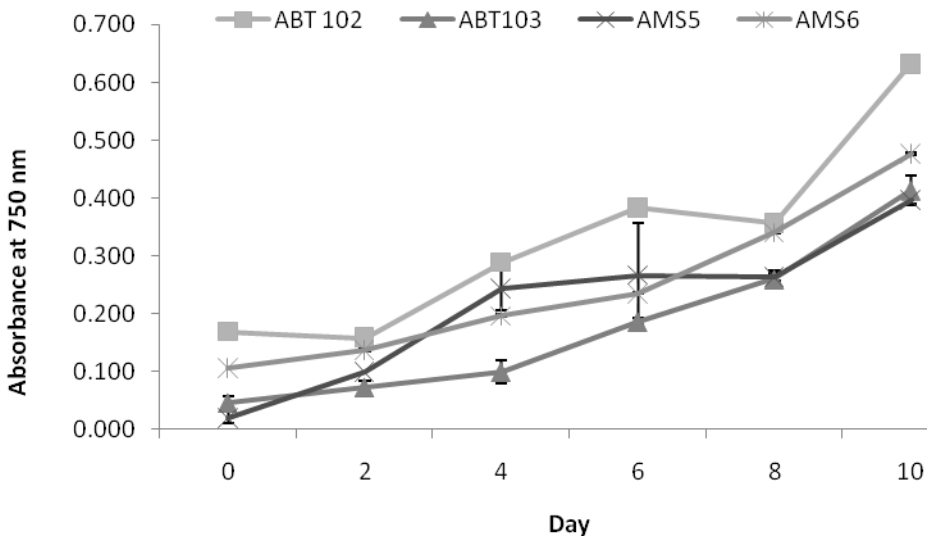


Fig.3 Growth dynamics of marine microalgae strains *Pavlova* sp. ABT102, *Chromulina* sp. ABT103, *Thalassiosira* sp. AMS 5 and *Chlorella* sp. AMS6 under laboratory conditions [A] OD [B] Dry weight

A



B

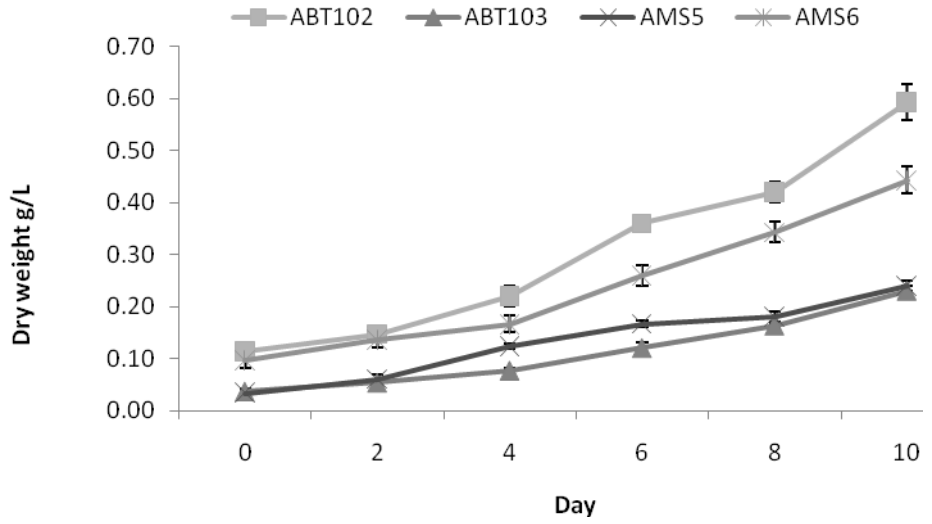


Fig.4 Effect of sodium bicarbonate on biomass and biochemical composition of *Pavlova* sp. ABT102, *Chromulina* sp. ABT103, *Thalassiosira* sp. AMS 5 and *Chlorella* sp. AMS6

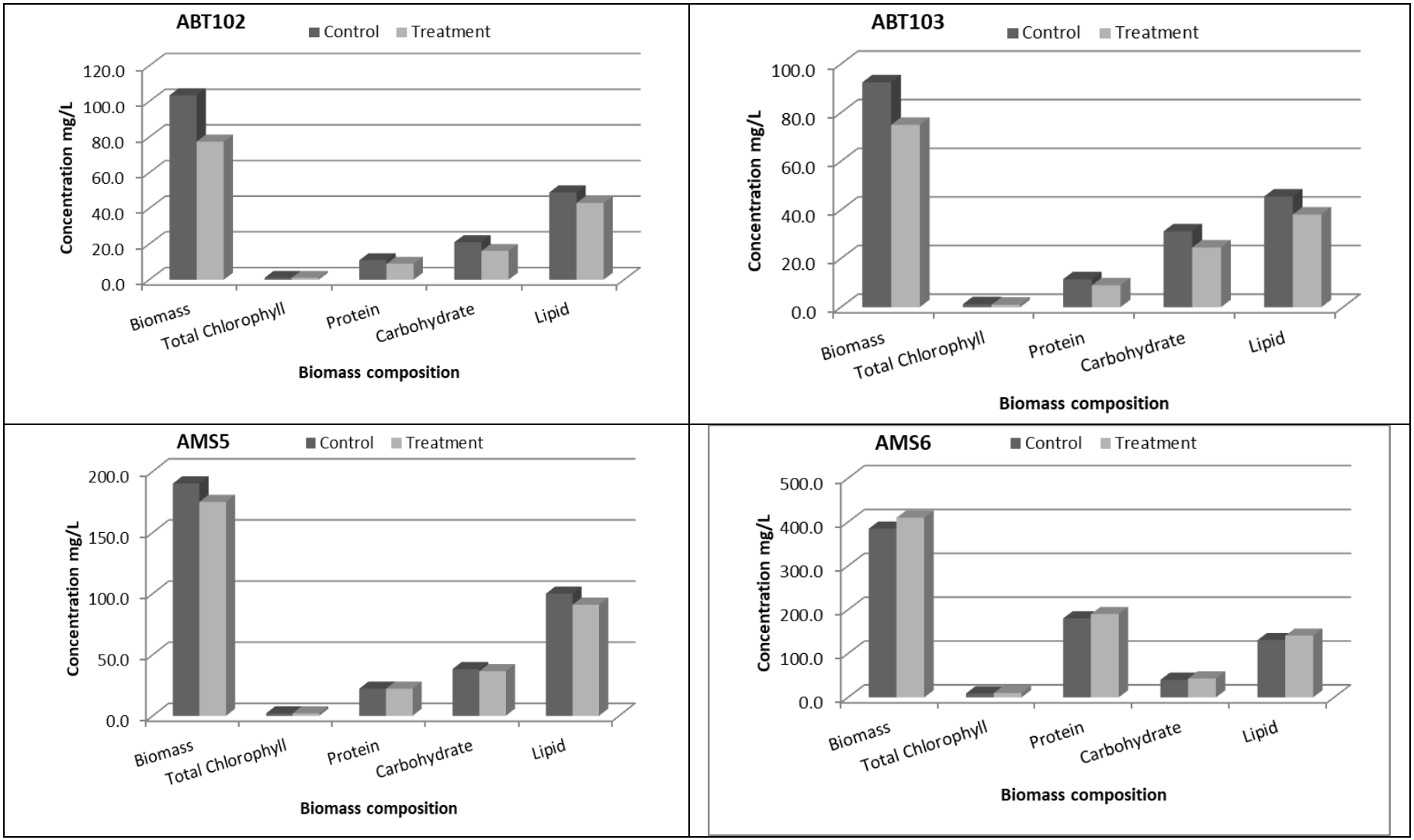
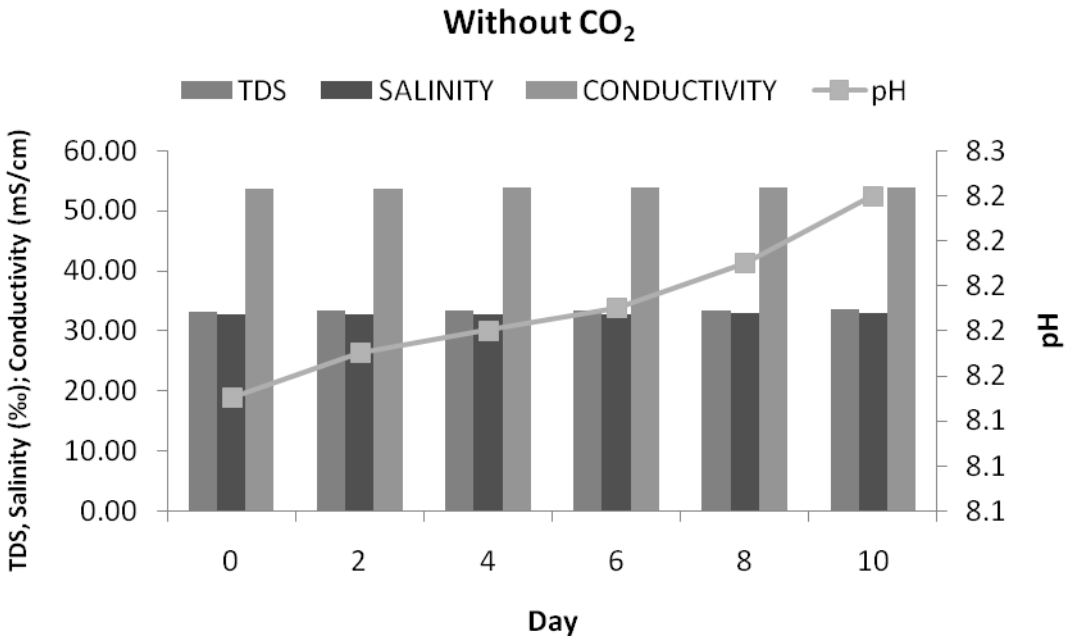


Fig.5 Physicochemical parameters of *Chlorella* sp. AMS6 grown in A (Without CO_2) and B (With CO_2) under open raceway pond at different intervals.

A



B

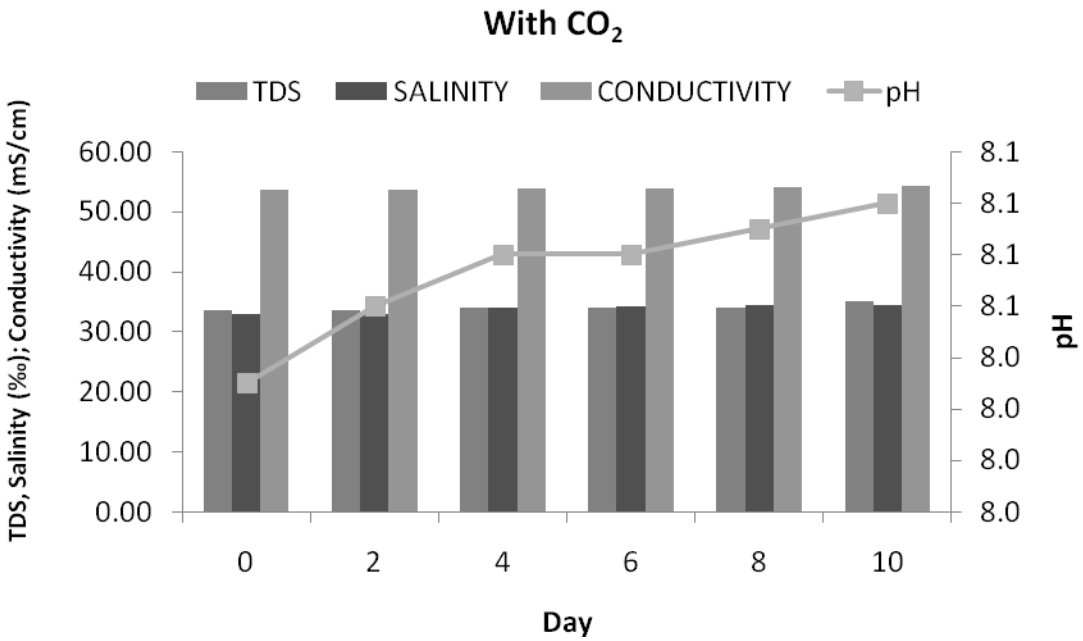


Fig.6 Growth dynamics of *Chlorella* sp. AMS6 with and without CO_2 in open raceway ponds at different time intervals [a] Absorbance [b] Dry wt

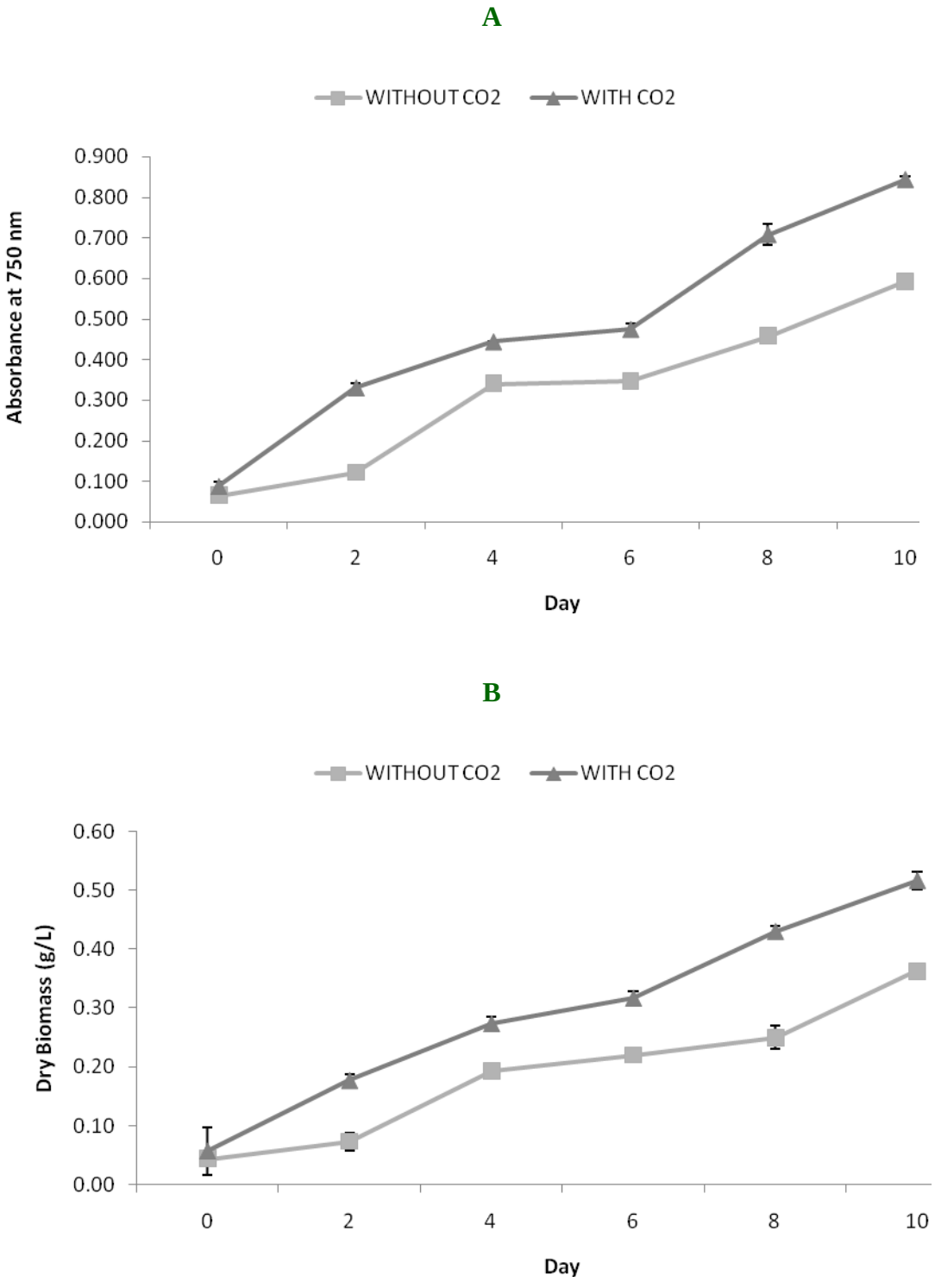
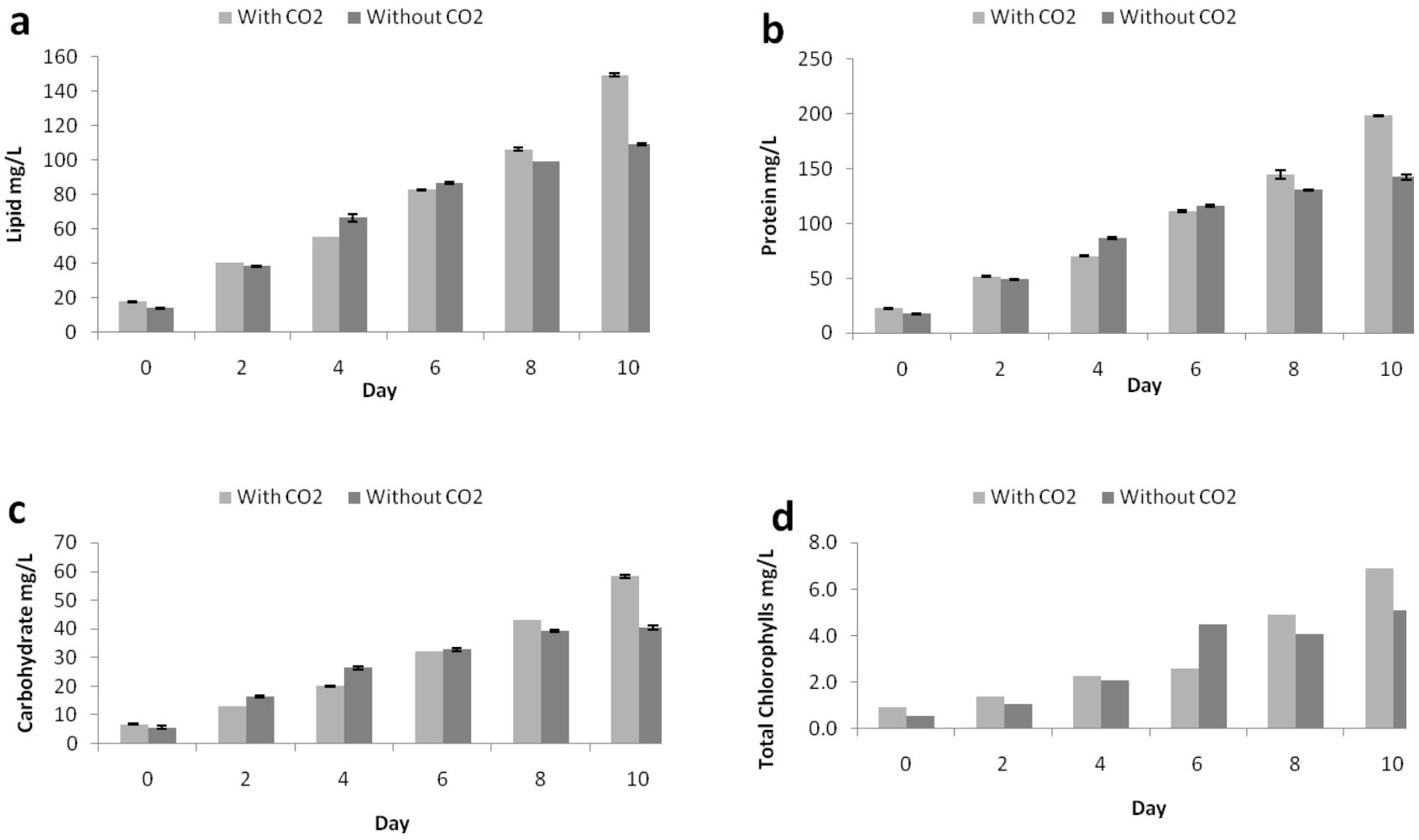


Fig.7 Changes in the biochemical composition of *Chlorella* sp. AMS6 with CO_2 and without CO_2 at different time intervals in 1.0 m^2 open raceway pond. [a] Lipid [b] Protein [c] Carbohydrate [d] Chlorophyll



Mathimani *et al.*, 2017 demonstrated contamination free, semi-continuous mode cultivation of marine *Chlorella vulgaris* BDUG 91771 at 5 kL open pond. Culture cultivated in seawater supplemented with inexpensive urea and superphosphate exhibited specific growth rate, biomass productivity, lipid content, lipid productivity, and doubling time of 0.703 d^{-1}, 0.056 g L^{-1} d^{-1}, 20% and 0.012 g L^{-1} d^{-1}, respectively at 30^{o}C average system temperature and 30 ppt salinity. In the present study though the strain recorded high lipid content, the volumetric biomass and lipid productivity with and without CO_2 supplementation recorded were lower than the values reported by Mathimani *et al.*, 2017.

Table 7 shows that the photosynthetic efficiency (PE) of *Chlorella* AMS 6 cultivated in 1 m^2 open raceway pond was 0.61% for the treatment supplemented with CO_2 and 0.43% for the control.

Biomass energy values or higher heating values (HHV) reported in the literature range from 20-23.75 KJ g^{-1} or MJ kg^{-133}. In this study, for the calculation of PE, we considered an energy value of 20 MJ kg^{-1} for the algal biomass. The treatment supplemented with CO_2 showed 42% increase in photosynthetic efficiency when compared to the treatment not supplemented with CO_2.

In the experiments conducted by Faria *et al.*, 2012 with addition of CO_2, the cell density of *Chlorella* sp. at the end of the experiments were 6.43 x 10^6 cell ml^{-1} in the control and 9.98 x 10^6 cell ml^{-1} in the treatment with addition of CO_2. It was opined that the variation in the cell density may be related to the availability of dissolved carbon to build several intracellular substances.

Chinnasamy et al., 2009 observed that the highest chlorophyll concentration and biomass of *C. vulgaris* ARC1 supplemented with 6% CO_2were 60 and 20 times more than that of *C. vulgaris* at ambient CO_2 (0.036%). *Chlorella* KR-1 species showed maximum growth at 10% CO_2 and good growth rate up to 50% CO_2 with a wide pH range and temperatures up to 40^{o}C (Sung *et al.*, 1999).

Biochemical composition

The volumetric and areal chlorophyll productivity of *Chlorella* ASM6 under CO_2 supplementation showed 36% increase over control. Similarly, the volumetric and areal productivities of lipids, carbohydrates and proteins in the treatment supplemented with CO_2recorded 38, 48 and 41% increase over control, respectively (Table 6). Except carbohydrates, the % of lipids, proteins and chlorophyll content of the alga in the treatment supplemented with CO_2 was 4.5, 2.6 and 5.3% less than the control. However, the volumetric and areal productivities of lipids, carbohydrates, proteins and chlorophyll in the treatment supplemented with CO_2 recorded 38, 49, 41 and 36% increase over the productivities obtained for the treatment not supplemented with CO_2.

In the present investigation, the protein content reported was between 38-40%, which was significantly higher as compared to previous studies. (Faria *et al.*, 2012) reported that the protein content ranging between 9-11% for *Chlorella* sp. in the control and 6-10% for the treatment supplemented with CO_2. Lourenço *et al.*, 2002 also found three times more protein in *Chlorella minutissima* than the protein content reported by Faria *et al.*, 2012.

Faria *et al.*, 2012 reported 19-61% carbohydrate content in *Chlorella* sp. in the cultures supplemented with CO_2 when compared to 20-26% reported for control. But, in the present study, the carbohydrate content reported for *Chlorella* sp. cultivated with and without CO_2 was about 11%. However, the lipid content observed in the present study was much higher i.e. 29-30% in both the treatments when compared to 4-13% as reported by Faria *et al.*, 2012.

The *Chlorella* strain used by Faria *et al.*, 2012 recorded low lipid% in the treatment supplemented with CO_2 when compared to the control. In the present study, there was no significant difference observed in the lipid, protein and carbohydrate % recorded for control and treatment supplemented with CO_2. Photosynthetic pigments are minor

substances in the chemical budget of microalgae which constitute less than 0.6% of the dry matter[38]. But in the present study, the total chlorophyll content observed in *Pavlova* sp. ABT 102, *Chromulina* sp. ABT 103, *Thallassiosira* sp. AMS 5 and *Chlorella* sp. AMS 6 were 0.67, 1.17, 0.77 and 1.41%, respectively.

Observations from the present study clearly indicate the vast diversity and genetic variation present in the algal strains with respect to biomass productivity and biochemical composition. This study also confirms that CO_2 addition could be a valuable strategy to significantly enhance the volumetric biomass and lipid productivities in algae.

Marine algae are potential sourcesof renewable feedstock for the production of food, feed, nutraceuticals, cosmeceuticals and green chemicals. In the present investigation, 4 different economically important marine microalgal strains such as *Pavlova* sp. ABT 102, *Chromulina* sp. ABT 103, *Thalassiosira* sp. AMS 5 and *Chlorella* sp. AMS 6 were screened for their biomass and lipid productivity through bench-scale studies. Though *Pavlova*sp. recorded highest volumetric biomass productivity followed by *Chlorella* sp. in the studies supplemented with inorganic carbon source, *Chlorella* recorded higher biomass productivity and lipid accumulation than the control when compared to the biomass and lipid values recorded for *Pavlova* sp.*Chlorella* sp. in the trials conducted at the outdoor open raceway ponds recorded higher volumetric and areal biomass and lipid productivities in the treatment supplemented with CO_2 when compared to the control. This study indicates the potential of various marine microalgae for the production of high value biomass as a source of value added compounds in the form of single cell proteins, carbohydrates and single cell oils which include omega fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) carbohydrates which can be used for food, feed and nutraceutical applications. This study also recommends identification and screening of potential marine algal strains and strain improvement and development of superlative strains through novel mutation breeding and gene editing using novel CRISPR/Prime editing approaches.

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Conflict of interest

All authors declare no conflicts of interest

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