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Effect of culture conditions on growth and lipid content of marine microalga *Nannochloropsis* sp. strain (PSDK11)

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Present study was aimed to estimate the growth, biomass and lipid production in *Nannochloropsis* sp. cultured under different culture mediums, pH and salinities. *Nannochloropsis* sp. (PSDK11) were isolated from Muthukuda mangrove water, Southeast coast of India and cultured in different mediums like Conway's, F/2, Miquel's, Schreiber's and TMRL, different pH conditions viz. 6.5, 7.0, 7.5, 8.0 8.5, under different salinities viz; 26, 28, 30, 32 and 34psu (practical salinity unit) for 18 days. Higher growth (0.721abs), biomass (8.0 g L^{-1}) and lipid production (19.12%) were achieved in Conway's medium, at 7.5 pH and 30psu of salinity.

[**Keywords:** *Nannochloropsis* sp., lipid, pH, salinity, biomass]

Introduction

Microalgae are rich in lipids, starch, and cellulose and are considered to be an important bioresource for generating a biodiesel[1]. In view of their high lipid content, they have received much attention as future raw materials for biodiesel synthesis[2]. Moreover algal based fuel production provide environmental benefits like decreased harmful emissions of carbon monoxide, hydrocarbons and hence the decreased greenhouse effect[3]. Several strategies have been adopted to improve the growth and lipid content of microalgae. These include optimization of the medium compositions (carbon source, vitamins, salts and nutrients), physical parameters (pH, temperature and light intensity), and type of metabolism (phototrophic, heterotrophic, mixotrophic and photoheterotrophic growth)[4-8].

Nannochloropsis also known as picopleustonic alga, belongs to the class Eustigmatophyceae, with the size range of 3-4 mm showing high lipid content[9] and hence being promoted as a potential source of lipid feedstock for biodiesel production[10]. Earlier studies focused on the optimization of light, salinity and nitrogen concentration [11], temperature[12], and CO_2[13] on growth and biochemical the influence of pH and media composition not yet to be studied in detail. Some earlier investigators have documented the influence of environmental factors on the biochemical profile of *Nannochloropsis*[14-17]. Present study focuses on the effects of different media, salinity and pH on the growth and lipid production of marine microalga, *Nannochloropsis* sp.

Materials and Methods

The microalgal samples were collected from the Palk Bay region of Muthukuda coast (Latitude: 9° 51' 48'' N; Longitude: 79° 7' 15'' E), Tamil Nadu, Southeast coast of India. Isolation and identification of microalgae was done by agar plating technique[18]. Cultures of *Nannochloropsis* sp. (Fig. 1) were grown in 500 mL flasks containing 300 mL of sterile

Conway's medium[19] under constant light conditions. The flasks were kept stable in an illumination incubator under cool-white fluorescent lights in 12:12 h light/dark cycles at 24±1℃. Cells in the late log-phase were isolated by centrifugation (4℃, 6000 ×g, 10 min), and the cell pellets were rinsed with double distilled water. Harvested cultures were freeze-dried, and the microalgae cells were stored at –20℃, prior to lipid analysis.

Table 1—Compositions of Conway's, F/2, Miquel's, Schreiber's and TMRL mediums

Compositions (Gram/Liter)	Conway's	F/2	Miquel's	Schreiber's	TMRL
Iron(III) Chloride Hexahydrate	1.30	-	-	-	3.0
Manganese(II) Chloride Tetrahydrate	0.36	-	-	-	-
Boric Acid	33.6	-	-	-	
Ethylene Diamine Tetraacetic Acid Disodium	45.0	4.36	-	-	-
(Sodium Dihydrogen Phosphate Dihydrate)	20.0	5.0	4.0	0.02	10
Sodium Nitrate	100	75.0	-	0.2	100
Potassium Nitrate	-	-	20.2	-	-
Sodium Metasilicate Nonahydrate	-	30.0	-	-	1.0
Zinc Chloride	2.1	-	-	-	-
Calcium Chloride	-	-	2.0	-	-
Cobalt(II) Chloride Hexahydrate	2.0	10.0	-	-	-
Ammonium Heptamolybdate Tetrahydrate	0.9	-	-	-	-
Copper(II) Sulfate Pentahydrate	2.0	9.8	-	-	-
Iron(III) Chloride Hexahydrate	-	3.15	2.0	-	-
Sodium Molybdate Dihydrate	-	6.3	-	-	-
Zinc Sulfate Heptahydrate	-	22.0	-	-	-
Manganese(II) Chloride Tetrahydrate	-	180.0	-	-	-
Hydrochloric Acid (ml)	-	-	2	-	-
Thamine Hydrochloric Acid	0.01	0.2	-	-	-
Cyanocobalmin	0.01	1.0	-	-	-
Biotin	0.0002	0.1	-	-	-

To determine the effects of culture media, the salinity, temperature, pH and light intensity were set at 30 psu, 24ºC, 7.5 and 200 µmol m^{-2} s^{-1} respectively. Culture media such as Conway's, F/2, Miquel's, Schreiber's and TMRL were used. Media compositions are presented in Table 1. To study the effect of pH, the temperature, salinity, light intensity and culture media were kept constant at 24ºC, 30 psu, 200 µmol m^{-2} s^{-1} and Conway's media respectively. The different pH such as 6.5, 7.0, 7.5, 8.0 and 8.5 were maintained by adjusting pH using either 1.0 mol L^{-1} HCl or NaOH.

To evaluate the effect of salinity, the temperature, pH, light intensity and culture media were maintained at 24ºC, 7.5, 200 µmol m^{-2} s^{-1} and Conway's respectively. Salinity was set at 26, 28, 30, 32 and 34 psu by adding the double distilled water in sterilized sea water.

The algal growth in the culture flasks were monitored every 24 hours by optical density measurement at a wavelength of 683 nm (i.e., OD_{683}) using a UV vis spectrophotometer (model 1800, Shimadzu, UV) after appropriate dilution with deionized water. Gravimetric method was used for algal biomass measurement according to Richmond *et al.*[20] with minor modification and method was explained in our previous publication[21]. Lipids were extracted according to the modified method of Bligh and dyer[10].

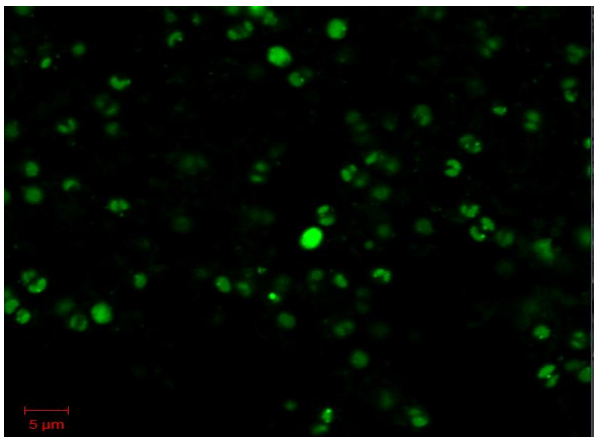

Fig. 1—Confocal micrographs of *Nannochloropsis* sp. (PSDK11).

Approximately 10 ml of wet algal biomass was mixed with methanol–chloroform (1:2 v/v). The mixture was then subjected to the microwave irradiation with 70% of power which was operated for 5 min at 65ºC. Reflux condenser played an important role to condense, return and maintain the solvent volume in the reaction mixture throughout the experiments. After the extraction reaction was completed, the chloroform–methanol phase that contains the extracted lipids was separated using a separating funnel followed by the evaporation of the solvent using rotary evaporator under vacuum condition. Total lipid (TL %) of the algal sample was determined gravimetrically by following equations TL % = (AEW-BEW) / WAS. Note: AEW – beaker weight after extraction; BEW – beaker weight before extraction; WAS - weight of the algal cell used for lipid extraction.

Results

The experiments were lasted for 18 days and maximum algal growth was observed at Conway's medium and cell density was 0.423 absorbance (abs) on 18th day of experiment (Fig. 2). During the first 9 days, growth rate of the alga in all culture medium was ~ 0.042abs. However, the final cell density was higher at day 18 in Conway's medium (0.423 abs) than that with F/2, Miquel's, Schreiber's and TMRL media where the cell growth was 0.211, 0.405, 0.171 and 0.414 abs respectively.

Figure 3 shows the biomass production of *Nannochloropsis* sp. (PSDK11) under different culture media and maximum yield of biomass (6.3 g L^{-1}) was obtained in Conway's medium and lower (2.2 g L^{-1}) biomass was found in Schreiber's medium on final day of the experiment (18th day). The biomass production was significantly lower ($P < 0.001$) in both Schreiber's (2.2 g L^{-1}) and F/2 media (3.2 g L^{-1}) when compared to the Conway's (6.3 g L^{-1}), Miquel's (4.6 g L^{-1}) and TMRL media (4.9 g L^{-1}). Significant differences were observed during the culture phases; the major dry biomass was observed at 9-15 days of the culture period (Fig. 3).

The *Nannochloropsis* sp. (PSDK11) was cultured for 18 days under a series of pH conditions (6.5, 7.0, 7.5, 8.0 and 8.5) and the results are being shown in Fig. 4. The maximum algal growth (0.512abs) was obtained at a pH of 7.5 and minimum was found in 8.5 (0.417abs). The pH range of 6.5 and 8.5 have shown significant effect on algae growth (P> 0.05) and the growth was 0.479abs, 0.497abs, 0.512, 0.477abs and 0.417abs at 6.5, 7.0, 7.5, 8.0 and 8.5 pH respectively.

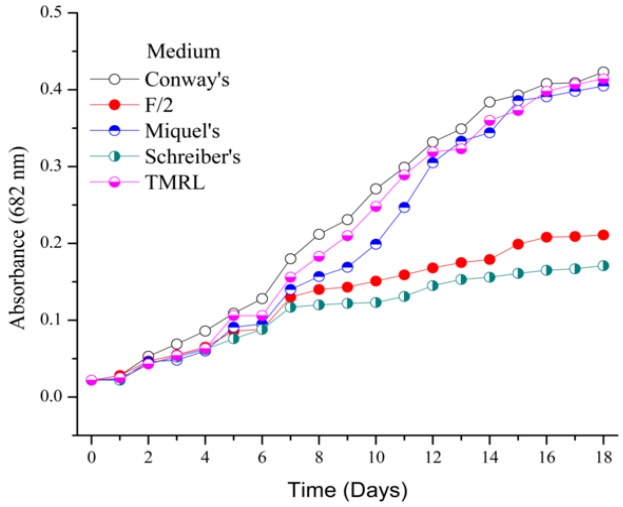

Fig. 2— Optical density (abs) versus culture period (day) for *Nannochloropsis* sp. (PSDK11) cultured in different medium (Conway's, F/2, Miquel's, Schreiber's and TMRL).

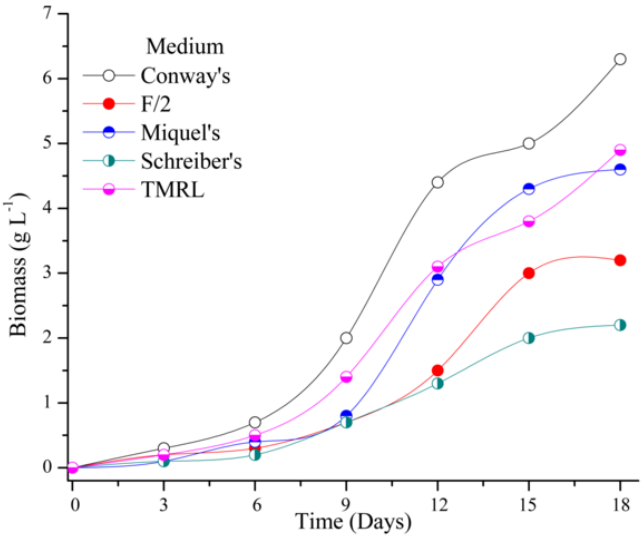

Fig. 3— Biomass production (g L^{-1}) versus culture period (day) for *Nannochloropsis* sp. (PSDK11) cultured in different medium (Conway's, F/2, Miquel's, Schreiber's and TMRL.

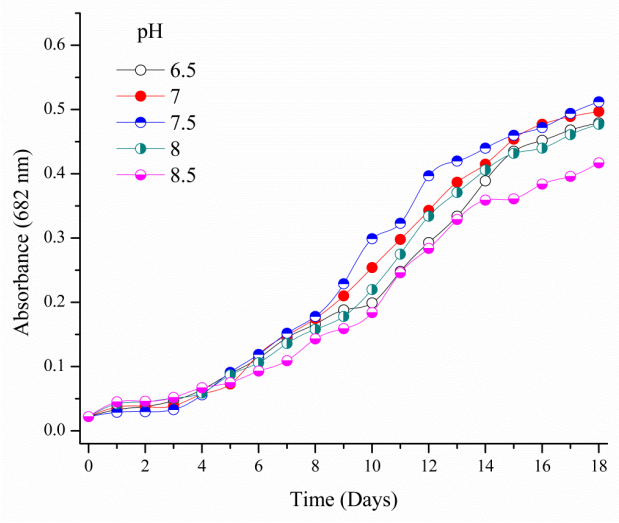

Fig. 4— Optical density (abs) versus culture period (day) for *Nannochloropsis* sp. (PSDK11) cultured in different pH (6.5, 7.0, 7.5, 8.0 and 8.5).

From the results in Fig.5, biomass production of *Nannochlorospsis* sp. (PSDK11) was significantly subdued at pH 7.5. Algal biomass was increased with increasing pH from 6.5 to 7.5 and decreased with increased pH from 7.5 to 8.5. The highest biomass production of 7.8 g L^{-1} was obtained at pH 7.5, and the lowest biomass production of 3.9 g L^{-1} was found at pH 6.5 (Fig.5). The best growth in *Nannochloropsis* sp. (PSDK11) has occurred at salinity 30psu with the highest growth rate of 0.721abs on the18th day of culture (Fig. 6) followed by 32, 28, 34 and 26psu with 0.680, 0.633, 0.415 and 0.386abs respectively.

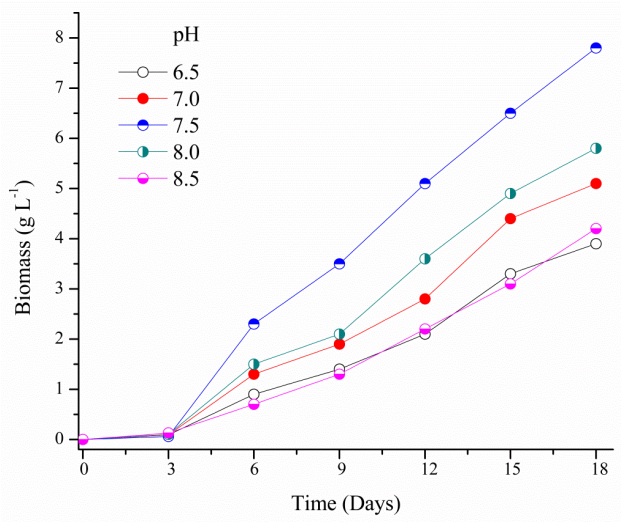

Fig. 5— Biomass production (g L^{-1}) versus culture period (day) for *Nannochloropsis* sp. (PSDK11) cultured in different pH (6.5, 7.0, 7.5, 8.0 and 8.5).

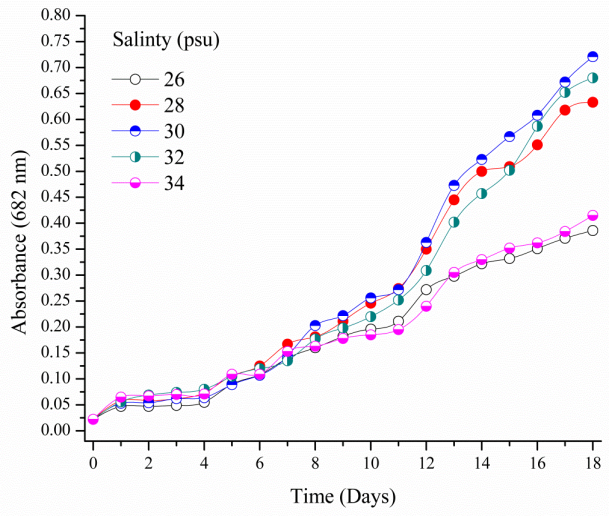

Fig. 6— Optical density (abs) versus culture period (day) for *Nannochloropsis* sp. (PSDK11) cultured in different salinity (26, 28, 30, 32 and 34psu).

Significant differences (P < 0.01) were found between salinity and algal growth. The effect of salinity on biomass production has been shown in Fig 7. Maximum biomass production (7.1 g L^{-1}) was found at 30psu of salinity and minimum (4.9 g L^{-1}) was in 26psu. Higher and lower salinity were resulted in low biomass when compared to the median salinity. The significant difference (P < 0.01) was found between biomass and salinity and the noticed trend was 8 g L^{-1} (30psu) > 7.6 g L^{-1} (32psu) > 7.1 g L^{-1} (28psu) > 5.3 g L^{-1} (34psu) > 4.9 g L^{-1} (26psu).

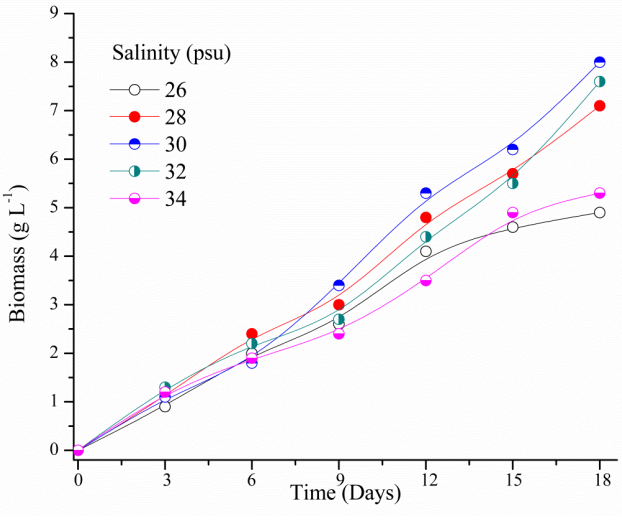

Fig. 7— Biomass production (g L^{-1}) versus culture period (day) for *Nannochloropsis* sp. (PSDK11) cultured in different salinity (26, 28, 30, 32 and 34psu).

Lipid profile of *Nannochloropsis* sp. (PSDK11) is shown in Fig. 8. The high lipid content of 7.84% was found in Conway's medium than the rest of the media tested (Schreiber's-3.52%, F/2-5.12%, Miquel's-7.36% and TMRL-6.88%). Similarly pH 7.5 result maximum lipid content (17.94%) and 30psu of salinity has shown maximum lipid yield (19.12%) (Fig. 8). *Nannochloropsis* sp. showed significantly higher ($p < 0.05$) lipid content when cultured in 30psu salinity, with 7.5 pH using Conway's medium compared to other parameters.

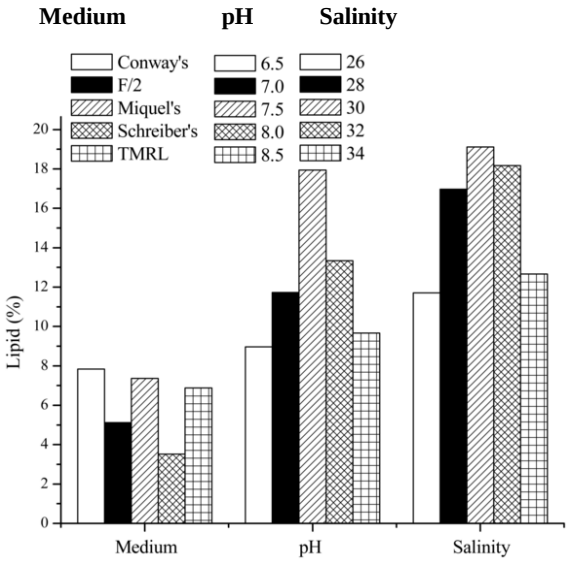

Fig. 8— Total lipid production of *Nannochloropsis* sp. (PSDK11) cultured in different medium (Conway's, F/2, Miquel's, Schreiber's and TMRL), pH (6.5, 7.0, 7.5, 8.0 and 8.5) and salinity (26, 28, 30, 32 and 34psu).

Discussion

Changes in environmental factors have performed influence in the growth of microalgae. Although many investigations were made in reference to many algae information pertaining to the *Nannochloropsis* sp. is very scarce[22-25]. In our study, the *Nannochlorpsis* sp. (PSDK11) culture through Conway's medium showed a higher growth, biomass and lipid production which would be due to the variety of chemicals of the Conway's medium compared to other media tested (Table 1). Rukminasari[12] and Chen et al.[26] have found that the growth rate and biomass production varied under different nutrient compositions. However, the lipid profile in microalgae has been often changed by nutrients depletion[5,27]. As regard, the biomass production, the second best production was found in TMRL medium (4.9 gL^{-1}) followed by Miquel's medium (4.6 gL^{-1}), but in lipid productivity second best result was obtained in Miquel's medium (7.36%) than with the TMRL medium (6.88%) (Fig.8). The findings showed that the lipid production rate could be due to the cell growth rate and biomass productivity[5].

The presently obtained pH based results are in line with the earlier reports[28, 29] who stated that the highest yield of biomass was achieved at the pH level of 7.5. The declining trend in biomass was observed in both decreasing and increasing pH from 7.5. Liu et al.[30] have found that, without changing pH (7.5 to 8.5) of seawater, *Chattonella marina* has produced higher biomass and its growth was declined when the pH was below 9. The concentration of CO_2, was available high in the medium when pH was 5 to 7.5. If pH increased, free CO_2 has been converted to bicarbonate and destroy the microalgae growth[31]. From this present investigation, it is understood that the *Nannochloropsis* sp. (PSDK11) cultured using Conway's medium at the pH 7.5 showed the maximum growth and biomass when compared to the other media and pH. The present study revealed that the negative log of the activity of the hydrogen ion in water can also made changes in lipid content of *Nannochloropsis* sp (PSDK11). Khalil et al.[28] also support our findings described that the biomass production and lipid content can increase when microalgae culturing in optimum pH (7.5). In the both treatments (medium and pH), it was found that *Nannochloropsis* sp. (PSDK11) produced highest lipid (19.12%) content when it cultured in optimized (Conway's medium with 30psu of salinity in pH 7.5) condition.

Salinity is an important environmental factor which plays an important role in algal production. In our observation, 30psu was found to be optimum salinity for the growth and biomass production of *Nannochloropsis* sp. (PSDK11) as opined by earlier workers[11, 32]. Present observation clearly demonstrated that the salinity affects both growth and lipid production in *Nannochloropsis* sp (PSDK11). The highest biomass and lipid proportion were procured at the salinity 30 (Fig. 7 and 8). Several studies were also reported our notion pointing that

higher salinity could be decreased the growth, lipid and fatty acids production[6, 33]. The main reason behind this is due to the ability of the organism to the optimized salinity conditions and an adaptation to the ambient osmotic pressure of microalgae[34]. Moreover, the growth of algae being to anemic at low salinity and algal cell division are low and therefore cell size may be increased[35].

Conclusion

The culture medium compositions, pH and salinity significantly influence the growth and biomass production of *Nannochloropsis* sp. (PSDK11). Higher growth (0.721abs), more biomass production (8.0 g L^{-1}) and elevated lipid content (19.12%) were achieved in optimized conditions of Conway's medium and with 7.5 of pH and 30 psu of salinity.

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