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GROWTH RATE, PIGMENT COMPOSITION AND BIOCHEMICAL ANALYSIS OF GREEN MICROALGAE, *D.OLIVACEUS* AND *C.HUMICOLA* IN FERTILIZER BASED OUTDOOR CULTIVATION

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ABSTRACT

Aim of the study: Media plays an important role in making the algal technology economical. To reduce the cost of the medium, an alternate source for nitrogen, phosphorus and potassium was used in the form of agricultural fertilizer (N:P:K in the ratio of 15:15:15). Materials and methods: The present study was conducted to determine the growth rate, pigment composition and biochemical analysis such as carbohydrate, protein and lipid content in fertilizer based outdoor cultivation (FBOC). Results and Discussion: Maximum growth rate, cell division rate and the yield was maximum for *Chlorococcum humicola* when compared to *Desmococcus olivaceus* in FBOC. Both the microalgae contains nonpolar substances includes carotenoids, chlorophylls and lipids reported to have potent antioxidant activity. Conclusion: Fertilizer based cultivation increased the accumulation of carbohydrates, protein and lipids in both the green microalgae.

Key words: Microalgae, Pigments, Carotenoids, Biochemicals, Fertilizer.

INTRODUCTION

Many cyanobacteria and microalgae were considered as natural source of various biologically and pharmacologically active compounds with structurally complex molecules which are difficult or impossible to be produced by chemical synthesis [1]. It is generally rich source of vitamins, essential amino acids, minerals, essential fatty acids such as γ linolenic acid and sulfo lipids [2]. Basically many of the metabolites produced by the organisms are in low amounts. The yield of algae was comparatively lower in laboratory culture. Nutrients required for growth of algae have been reported to be from organic and inorganic sources .In order to achieve optimum growth, nutrient should be adequate in quantity [3,4]. Apart from carbon, hydrogen and oxygen algae require additional compounds to grow like nitrates, phosphates and sulphates which are important for vital functions in algae. Hence the present work was carried out to study the growth rate, pigment composition and biochemical analysis of green microalgae, *Desmococcus olivaceus* and *Chlorococcum humicola* in fertilizer based outdoor cultivation.

MATERIALS AND METHODS

Collection of microalgae

Freshwater green microalgae, *Desmococcus olivaceus* and *Chlorococcum humicola* were obtained from the Vivekananda Institute of algal Technology (VIAT), Chennai.

Fertilizer based Outdoor Cultivation of Microalgae

Chlorococcum humicola and *Desmococcus olivaceus* were grown in improvised CFTRI medium [5] Fertilizer based medium is a simple medium containing commercial fertilizer (N: P: K in the ratio of 15:15:15) as a

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supplemental of nitrogen, phosphorus and potassium was used in the cultivation of microalgae. 500ml of micro algal inoculums was added in to 20 l of ground water containing 0.25g / l of NPK fertilizer and grown with a facility to pump the culture with air. Algal cultures were grown for 15 days with fertilizer based medium in outdoor conditions in 20 L tank, the pH of the medium should be maintained between 8-10 pH.

Measurements of growth

Growth parameters included cell counts, fresh weight (FW) and dry weight (DW). The cell counts were determined using haemocytometer slide [6]. Fresh weight of cells was taken after filtering and removing the excess of moisture using blotting paper. Dry weight of cells was taken after filtering and drying overnight at 60ºC.

Pigments

Chlorophylls, carotenoid and total pigment were determined according to Lichtenthaler [7]. Pigments are identified using their absorption maxima by spectrophotometry. Methanol extracts of *C.humicola* and ethyl acetate extracts of *D.olivaceus* are spotted on silica gel TLC readymade plates and separated using solvent system Acetone: Hexane: Ethyl acetate. Standard β carotene was spotted on the TLC plate and Rf values for each spot were calculated and compared with the standard (β carotene).

Proximate composition

Moisture and ash content were determined according to AOAC [8]. Total carbohydrate, total protein and total lipids were determined by Anthrone method [9], Lowry method [10] and Bligh and Dyer [11] respectively.

RESULTS AND DISCUSSION

The growth of microorganisms was significantly increased in fertilizer based outdoor cultivation. Maximum cell count attained in culture was up to 224×10^4 cells and 250×10^4 cells in *D. olivaceus* and *C.humicola* respectively in 15 days. Maximum cell count was achieved in *Chlorococcum* which was significantly more than *D. olivaceus* (Figure 1).

In the present study, approximately 1000 l culture needed to be centrifuged to yield wet biomass which constitutes 3.23 and 3.28 g/l for *D. olivaceus* and *C.humicola*, respectively. Dry weights of *Desmococcus* and *Chlorococcum* were 0.30 and 0.34 g/l in 15 days (Figure 2). Maximum growth rate, cell division rate and the yield was maximum for *C. humicola* when compared to *D.olivaceus* in fertilizer based medium. The stimulation of growth and yield of *D.olivaceus* and *C.humicola* may be attributed to induction of some metabolic enzyme activities has been noted for *Dunaliella tertioleca* [10]. The algal biomass obtained was subjected for quantification of carotenoids and chlorophyll pigments. *C. humicola* showed high amounts of chlorophyll and carotenoid pigments (0.65 and 0.47 mg/g) respectively (Table 1). Both carotenoids and chlorophyll were identified using their characteristic absorption maxima, carotenoids exhibited peak, a major peak at 535 nm and a peak at 608 and 664 nm for chlorophyll in *D. olivaceus* .In *C.humicola*, three peaks were observed at 410, 611 and 663 nm respectively for carotenoids and chlorophyll (Figure 3 a & b).

Identification of carotenoids and chlorophyll by TLC

The extract of each band obtained in TLC was analysed, which showed similar profile of carotenoid peaks except they differed in their relative quantities of β-carotene fraction (Figure 4). Rf values of pigments of microalgae *D.olivaceus* and *C.humicola* are depicted in Table 2. Some of the spots identified in *Chlorococcum* showed maximum amount of pigments (chlorophyll and carotenoids), when grown with N:P:K as fertilizer in the medium are β carotene (Rf 0.93), Zeaxanthin (Rf 0.54), Lutein (Rf 0.23) and Chlorophyll a and b (Rf 0.32 and 0.27) respectively.

Biochemical analysis of *D. olivaceus* and *C. humicola*

Biochemical analysis of *D.olivaceus* and *C.humicola* showed the presence of high protein and ash. Other constituents of algae include carbohydrate, fat and moisture was seen in both the algal forms (Figure 5). Protein and lipid contents were maximum in *C. humicola* when compared to *D olivaceus* .The obtained results revealed that in *C. humicola*, protein is major organic constituent (26%) followed by lipid and carbohydrates (14.26% and 11.4% respectively), whereas for *D.olivaceus* protein is major constituent (21%), with lipid (10.31%) and carbohydrate (11.86%). The moisture and ash content are more in *D.olivaceus* when compared to *C.humicola*. Nutraceutical compounds were recorded in blue green algae, *Spirulina max* and marine algae by Hanaa *et al* [12] and Ajit Kandale *et al* [13]. In plants and algae, carotenoids serve both photosynthetic and photo protective role.

Holick [14] and Rock [15] reported that naturally occurring carotenoids, other than β carotene have exhibited anticancer activity and are being considered further as potential chemopreventive agents. Nonpolar substances include carotenoids (β-carotene and Zeaxanthin), Chlorophyll and fattyacids which were largely enhanced by salinity stress and reported to have higher antioxidant activity [16,17].

Table 1. Growth and pigment composition of *D .olivaceus* and *C. humicola*

S.no	Contents	*D.olivaceus*	*C.humicola*
1	Cell count ($\times 10^4$cells/ml)	224 ± 20	250±25
2	Growth rate (divisions/day)	0.32±0.14	0.38±0.03
3	Biomass fresh weight (g/l)	3.23±0.25	3.28±0.35
4	Biomass dry weight (g/l)	0.30±0.06	0.34±0.02
5	Total chlorophyll (mg/g)	0.47±0.04	0.65±0.05
6	Total carotenoid (mg/g)	0.21±0.03	0.47±0.05

Table 2. Rf values of different pigments of microalgae, *D.olivaceus* and *C.humicola*

Sl.No.	Possible Compound	Rf
1	Synthetic β carotene	0.93
2	β carotene	0.93
3	Zeaxanthin	0.54
4	Chlorophyll a	0.32
5	Chlorophyll b	0.27
6	Lutein	0.23

Figure 1. Growth rate of *Desmococcus olivaceus* and *Chlorococcum humicola*

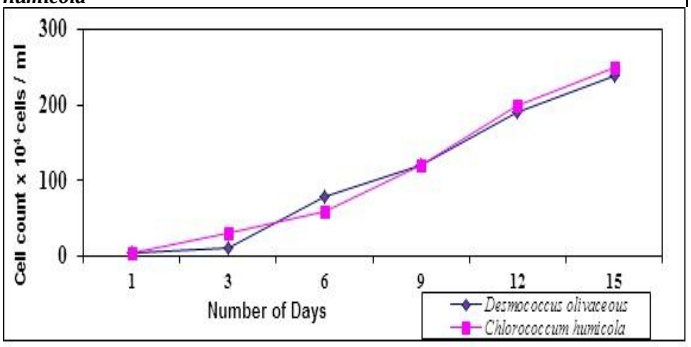


Figure 2. Dry and fresh wet weight of *Desmococcus olivaceus*and *Chlorococcum humicola*

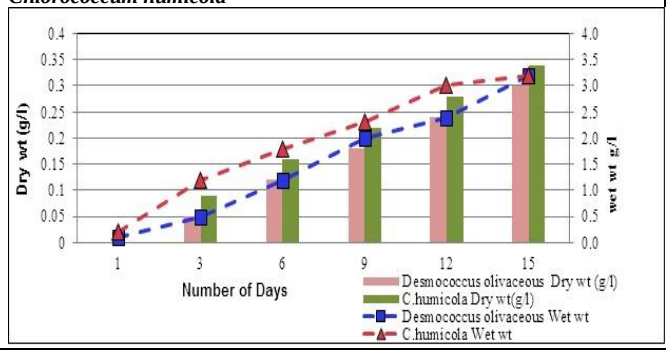


Figure 3 a & b: UV VIS Spectra of *Desmococcus olivaceus and Chlorococcum humicola*

Figure 3a

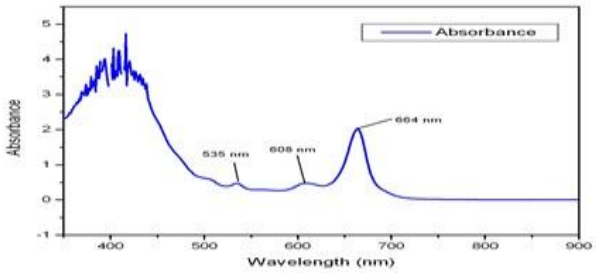


Figure 3b

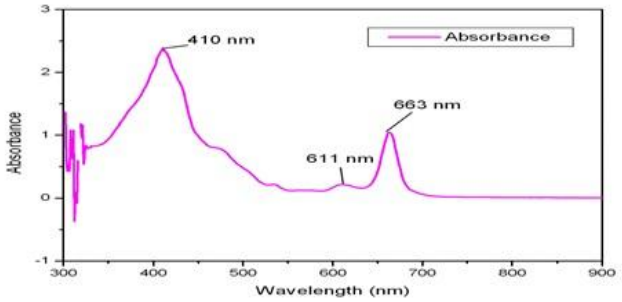


Figure 4. TLC showing the presence of pigments in *D.olivaceus* and *C.humicola*

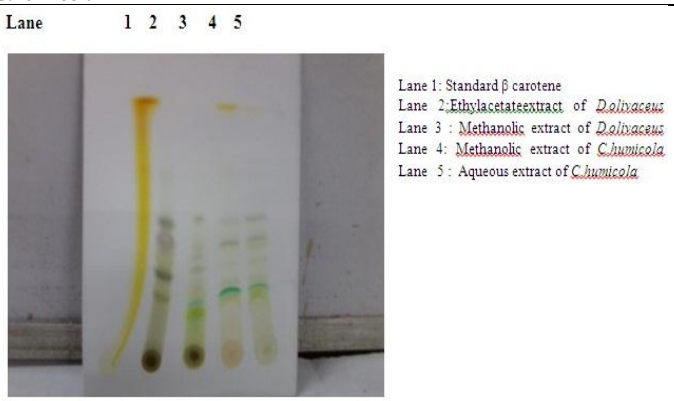
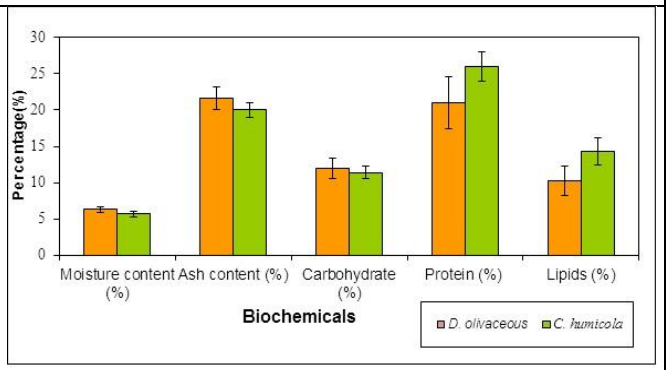


Figure 5. Proximate composition of *D.olivaceus* and *C.humicola*



(Values are mean ±SD (n=3)

CONCLUSION

Cultivation of green microalgae in fertilizer based medium outdoor cultivation, led to alteration of algal metabolism as well as an enhancement or induction of bioactive compounds. The present study indicated that maximum growth rate and yield was observed in *D.olivaceus* and *C.humicola* grown in outdoor cultivation. Micro algae also contains high amount of pigments and biochemicals which are required for dietary supplements and of nutritional value.

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