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EFFECT OF GROWTH PHASE IN ENHANCING PUFA ACCUMULATION OF FEW OLEAGINOUS FRESHWATER CHLOROPHYCEAE SPECIES

Susmita Das[1], Ajitabh Bora[1*], Hemanta K Gogoi[1], Sushil Manderia[2], Barnali Bailung[1], Jadab Rajkonwar[1] and Sanjai K. Dwivedi[1]

[1]Defence Research Laboratory, P.B. No.-2, Tezpur-784001, Assam
[2]SOS in Botany, Jiwaji University, Gwalior, M.P

***Corresponding Author Email: ajitabhbora@gmail.com**

ABSTRACT

Freshwater green microalgae are potential sources of vegetarian PUFAs. The fresh water bodies harbor diverse forms of microalgae, which have hitherto remained untapped. With an aim to tap these resources, few green microalgae were evaluated and compared with the different growth phase for their PUFA composition increment with a special interest in omega-3 and omega-6 fatty acid. PUFA content of seven algal species was analyzed by culturing in BG11 medium rich in nitrogen source and grow under unique growth condition established in the laboratory (unpublished). The test species accumulated maximum lipid content during stationary phase when grown in batch mode. GC-MS analysis of lipid extracted during log phase revealed presence of only Linoleic acid, while during stationary phase, other PUFAs were detected such as Docosahexaenoic acid (C22:6) and Stearidonic acid (C18:4) in Chlorococcum humicola, Arachidonic acid (C20:4) in Ankistrodesmus falcatus, Eicosadienoic acid (C20:2) in Desmodesmus opliensis, Hexadecatrienoic acid (C16:3) in Chloralla ellipsoidea and Hexadecatetraenoic acid (C16:4) in Coelastrum microporum. This study thus established a novel growth condition and particular growth phase in which PUFA accumulation enhanced in test fresh water microalgae.

KEY WORDS

Microalgae, PUFA, BG11, Omega 3 and 6 fatty acid

1. INTRODUCTION

Alga is recognized as one of the oldest forms of life on earth. Members of the Chlorophyceae (green algae) are presently targeted as a potential candidate for the production of Nutraceuticals such as omega -3 fatty acids [1] and also as a food for human and animal consumption [33]. Microalgae chiefly are made up of protein, carbohydrates, and lipids and are a potential non-conventional source of nutraceutical compounds.

Currently, nutritional research focuses are on the non-conventional source of nutrition and algae have proved to be one of the best sources [33]. Algae can hoard energy in the form of oils and carbohydrates, which, when pooled with their high productivity, can produce from 2,000 to as many as 5,000 gallons of oil per acre per year [9,16, 20].

Microalgal lipids are composed of unsaturated fatty acids (7 to 22 carbon atoms), among which omega-3 and omega-6 families are of particular interest such as eicosapentaenoic acid (EPA), arachidonic acid (AA) [33]. They are collectively called polyunsaturated fatty acids (PUFAs) which form an essential component of membrane phospholipids [6] as well as stored lipids [12] besides several beneficial roles in the normal functioning of metabolism [29]. Currently, fish and fish oil are the only sources of long- chain PUFAs. Unpleasant fishy smell and poor oxidative stability are two factors which make fish and their products

unpopular for consumption [5, 33]. Commercially available algal lipid is EPA, which is nutritionally important for the cardiovascular and brain function in higher eukaryotes [21, 31, 39] and mostly marine algae are the chief source of PUFAs [2].

EPA and DHA are found in different marine species [2, 37]. Docosahexaenoic acid is primarily found in Dinophyceae and Prymnesiophyceae [18, 22]. The species which are producing long-chain polyunsaturated fatty acids show low levels of C18 fatty acid precursors [13]. Microalgae species with high lipid contents of 30–70 % dcw has been reported [41]. The search for non-conventional sources of PUFAs has progressed in recent years with special interest in EPA and DHA [14].

The fresh water microalgae have almost remained unexplored for nutraceutical applications. In this study, we tried to show the effect of two stages of growth viz. log and stationary phase on total lipid and PUFA content increment of seven fresh water green micro algal species.

2. MATERIALS AND METHODS

2.1 Cultures and cultivation

Seven numbers of freshwater green microalgal species viz. *Ankistrodesmus angustus* (aa), *Ankistrodesmus falcatus* (af*), Chlorella ellipsoidea* (ce), *Desmodesmus opoliensis* (do), *Chlorococcum humicola* (ch), *Coelastrum microporum* (cm), *Scenedesmus bijugatus* (sb) was collected from Algae Culture Facility of Defense Research Laboratory, DRDO, Tezpur, Assam. The cultures were first grown in modified BG-11 medium containing 1.5 g/l Sodium nitrate (Table I &II) and was reinoculated in modified BG-11 media in their log phase in fresh medium at a mass fraction of 5%. The cultures were grown in 500 ml flask in batch culture mode and were maintained *in vitro* at 25±2°C with photon flux density of 45 µ Mol m^{-2} s^{-1} under a photoperiod of 16:08 hours light and dark cycle. This condition was preliminarily studied in the laboratory and found optimal for algal growth (unpublished)

Table I: Major components of BG11 media

Component	Stock solution (g/100ml)	Aliquot (ml/L)
K_2HPO_4	3.13	1
Na_2CO_3	2	1
$MgSO_4.7H_20$	7.40	1
$CaCl_2.2H_20$	3.60	1
EDTA	0.10	1
Citric acid	0.60	1
Ferric ammonium citrate	0.60	1
Trace metal solution		1

Table II: Major component of trace metal solution

Component	Stock solution (g/100ml)
H_3BO_3	**0.003**
$MnCl_2.4H_2O$	**0.18**
$ZnSO_4.7H_2O$	**0.022**
$Na_2MoO_4.2H_2O$	**0.039**
$Co (NO_3).6H_2O$	**0.005**
$CuSO_4.5H_2O$	**0.008**

2.2.1. Analysis of growth rate

Cell growth was determined by measuring the absorbance at 680 nm using UV/VIS spectrophotometer (Thermo) up to 42 days.

2.2.2. Estimation of biomass

Dry cell weight (dcw) was determined gravimetrically [30]. 50 ml of algal culture was centrifuged at 5000 RPM for 10 minutes and the harvested biomass was dried at

60°C till it reached a constant weight. The specific growth rate attained during exponential growth was determined by using the following equation:

$\mu e = (\ln BC_2 - \ln BC_1)/(t_2 - t_1)$

Where, µe is the specific growth rate (mg $mL^{-1}d^{-1}$); and BC_2 and BC_1 are, respectively, the biomass concentrations (mg mL^{-1}) at times t_2 and t_1 (in days).

2.2.3. Analysis of Chlorophyll content

2 ml of algal culture was centrifuged at 7000 RPM for 5 minutes. The harvested biomass were subjected to methanol extraction for overnight at 4°C. The suspension is centrifuged at 5000 RPM and the supernatant was transferred into a new vial. The concentration of the chlorophyll content is determined spectrophotometrically by using the following equation [24]:

Chlorophyll A = $12.7A_{663nm} - 2.69A_{645nm}$

Chlorophyll B = $22.9\ A_{645nm} - 4.86\ A_{663nm}$

A_{663nm} = Absorbance at wavelength of 663nm.

A_{645nm} = Absorbance at wavelength of 645nm.

Total Chlorophyll Content = Chlorophyll A+ Chlorophyll B

2.2.4. Estimation of Carbohydrate

100 mg of freeze- dried sample was hydrolyzed by keeping it in a water bath for 3 hours with 5 ml of 2.5 N HCl and then neutralized with Sodium carbonate until the effervescence ceases. The volume was made up to100 ml and centrifuged at 5000 RPM for 10 minutes. The supernatant was collected, and 1 ml aliquot was used for analysis by Phenol-Sulphuric method [10]. The reading was taken at 490 nm. The concentration of carbohydrate in the sample was calculated from a calibration curve prepared by using glucose (Sigma, USA) as a standard.

2.2.5. Estimation of Protein

50 mg of freeze- dried sample was incubated in 1 ml of Ultrapure water overnight at 4 ^{0}C. The sample after incubation was homogenized for 5 minutes and then centrifuged for 20 minutes at 4 ^{0}C at 15000g. The supernatant was collected and kept on ice. 1 ml of 1N NaOH was added to the pellet and kept at room temperature for 1 hour with occasional shaking. The protein content was measured by the Lowry method [23]. The concentration of protein in the sample was calculated from a calibration curve prepared by using Bovine Serum Albumin (BSA fraction 5, Sigma, USA) as a standard.

2.2.6. Rapid estimation of lipid by Nile red fluorescence method

2 µl of Nile red (20 µg Nile red L^{-1} acetone) was added to 2 ml of fresh algal sample having a cell density of 2×10^4 to 10×10^5 cells/m L. The suspension was incubated for 20 minutes in the dark at room temperature. The relative fluorescence was measured at excitation and emission wavelengths of 530nm and 575nm respectively in a Fluorescence spectrophotometer (Perkin Elmer) and non-stained cells in the medium were used as a control.

2.2.7. Extraction of Total lipid and their analysis

500 mg of freeze-dried algal sample was used for extraction of total lipid following Bligh and Dyer method [3]. The extract was distilled in a rotary vacuum evaporator at ≤50 °C. The quantity was measured gravimetrically and expressed as dry weight %. The lipid samples were stored at 4 °C for further analysis.

2.2.8. Analysis of fatty acid

The fatty acid profile was determined through GC-MS analysis of fatty acid methyl esters (FAME) of the algal lipids. FAME was prepared by, using a fraction of hydrochloric acid in methanol. The top organic phase was extracted and 2 µl of it was injected into Agilent 7890 series Gas chromatography fitted with 19091J-413 capillary column (30m x 320um x 0.25µm) at the injection temperature maintained at 250 °C with a split ratio of 50:1. The gas (helium) flow rate was 1.4 ml/min. The oven temperature was initially set at 50 °C for 1 minute and later increased to 7 ^{0}C/min to 250 ^{0}C for 5 minutes. FAMEs were identified by comparing their fragmentation pattern and retention index with NIST library. The standard used to be 37components FAME mix (Sigma, USA).

2.2.9. Statistical analysis

One-way ANOVA test ($p < 0.05$) was used for data analysis and least significant differences between treatment means were determined by Duncan's multiple-range test ($p < 0.05$) [43].

3. RESULT AND DISCUSSION

3.1. Growth and Biomass Production

Good growth of all the seven microalgae cultured in BG11 medium was observed. Maximum cell density and biomass were recorded in *Chlorella ellipsoidea* (ce) and lowest in *Ankistrodesmus falcatus* (af) (Fig.3.1). Toyub [38] reported that *C. ellipsoidea* was producing 629.13 mg L^{-1} biomass in BBM media, which is much less than

our report in BG11 media rich in nitrogen. *C. pyrenoidosa* produced maximum biomass and xanthophylls when grown in BG11 medium containing urea as a nitrogen source [35,36]. Thus, we observed that nitrogen is a vital component for the growth of oleaginous microalgae. Among all the species, *Chlorella ellipsoidea* showed maximum biomass yield in our study. The biomass concentration of *Chlorella ellipsoidea* was 0.93 mg mL^{-1} and specific growth rate was 0.146 mg $mL^{-1}d^{-1}$ while in case of *Ankistrodesmus falcatus* biomass concentration was 0.82 mg mL^{-1} and specific growth rate was 0.036 mg $mL^{-1}d^{-1}$.The biomass concentration of *Ankistrodesmus angustus, Desmodesmus opoliensis, Chlorococcum humicola , Coelastrum microporum, Scenedesmus bijugatus* was 0.92mgmL^{-1},0.91mgmL^{-1},0.85 mgmL^{-1},0.87 mgmL^{-1},0.83 mgmL^{-1} and their specific gowth rate was 0.126 mg $mL^{-1}d^{-1}$,0.05 mg $mL^{-1}d^{-1}$,0.042 mg $mL^{-1}d^{-1}$,0.046 mg $mL^{-1}d^{-1}$,0.039 mg $mL^{-1}d^{-1}$.. From the graph it was clearly expressed that the lag phase is from 0 to 6 days while log phase varies from 7 days to 28 days in all species except *Desmodesmus opoliensis* and *Chlorella ellipsoidea*. In *Desmodesmus opoliensis* log phase is from 7days to 22 days and *Chlorella ellipsoidea* it was from 7 days to 24 days and stationary phase continue up to 38 days in all the species and after that decline phase of algae start.

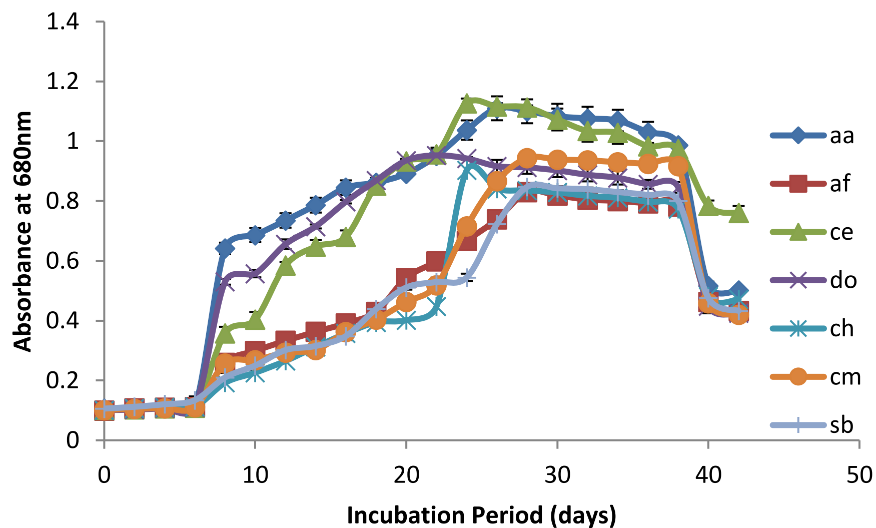

Fig. 3.1 (A): Growth curve of seven species in BG11 medium. Bars indicate mean ± SE of four replicates.

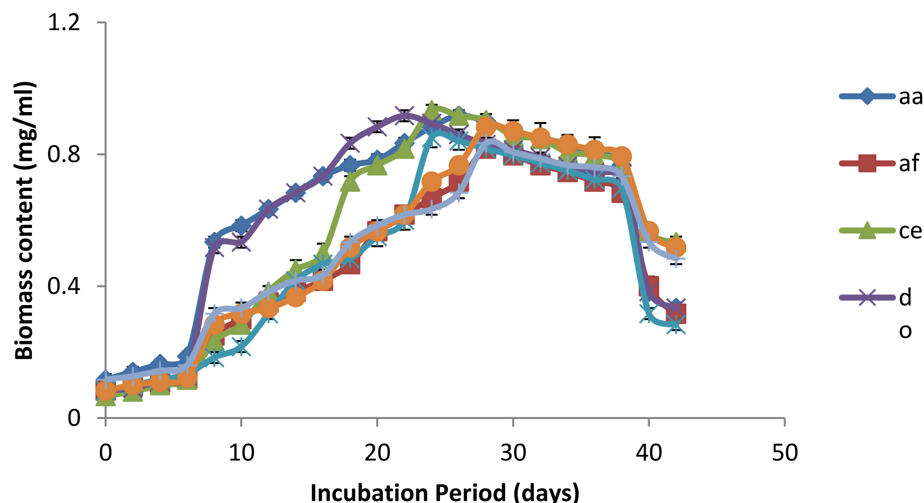

Fig.3.1 (B): Biomass content of seven species in BG11 medium. Bars indicate mean ± SE of four replicates

3.2. Analysis of Chlorophyll Content

The chlorophyll content of the seven test microalgal species is shown in Fig.3.2. From the figure, we can observe that on the log phase of culture, chlorophyll content was highest in *C. ellipsoidea* (38.443mg/L) and lowest in *A. falcatus* (11.46 mg/L). The chlorophyll content of *Ankistrodesmus angustus, Desmodesmus opoliensis, Chlorococcum humicola , Coelastrum microporum, Scenedesmus bijugatus* in their log phase was 36.43 mg/L,20.7 mg/L,13 mg/L, 16.45 mg/L,12.78 mg/L. From the results we observed that, the chlorophyll content of the test algae was highest in log phase, which gradually decreased in stationary and decline phase in batch culture.

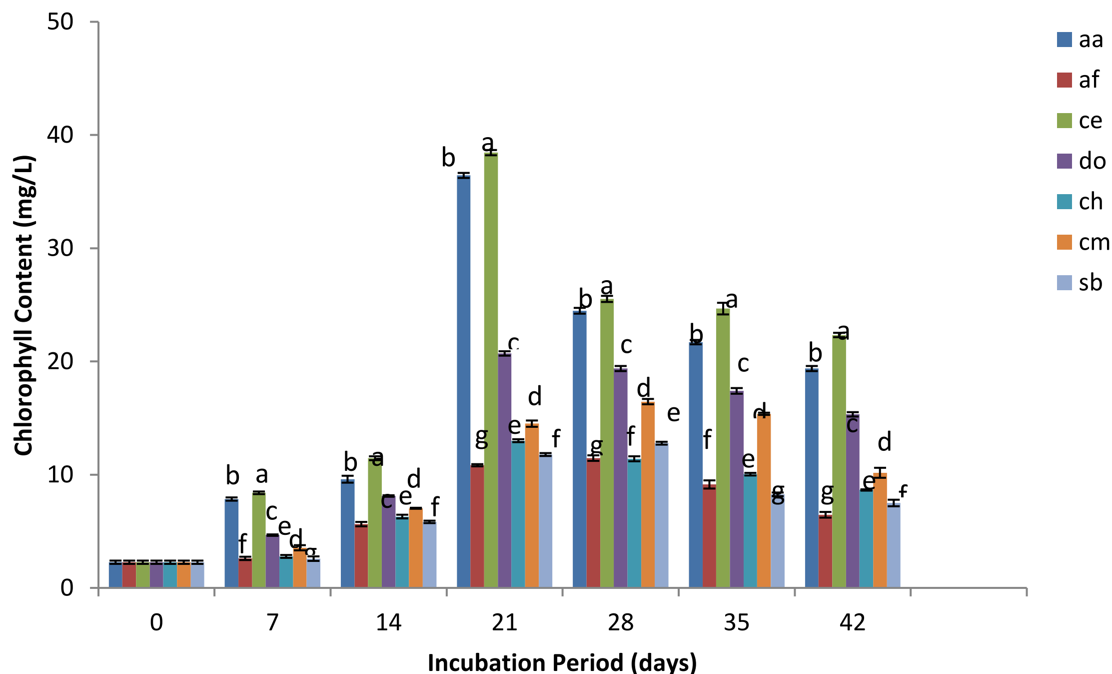

Fig.3.2: Chlorophyll contents of seven micro algal species in BG11 medium. Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test(Zar et al.(1984))[43].

3.3 Analysis of Carbohydrate

The carbohydrate content of the seven-test micro algal species cultured in BG11 media is shown in Fig.3.3. Mohan [27] showed a parallel trend of gradual increase in carbohydrate accumulation throughout the study period in *C. vulgaris*. From the result, it is clear that the accumulation of carbohydrate occurs in stationary phase. In stationary phase, cells are in stress condition and hence during the biochemical cycle, acetyl CoA may not enter into the Krebs cycle to release stress [32,18].

The carbohydrate concentration increased steadily in *Ankistrodesmus angustus* and *Scenedesmus bijugatus* in stationary phase reaching a maximum of 64% dcw which was highest among seven species and the lowest concentration of carbohydrate was found in *Desmodesmus opliensis* (51% dcw) among seven species for the same period. The details of carbohydrate content in all the species in stationary and log phase were given in Table III.

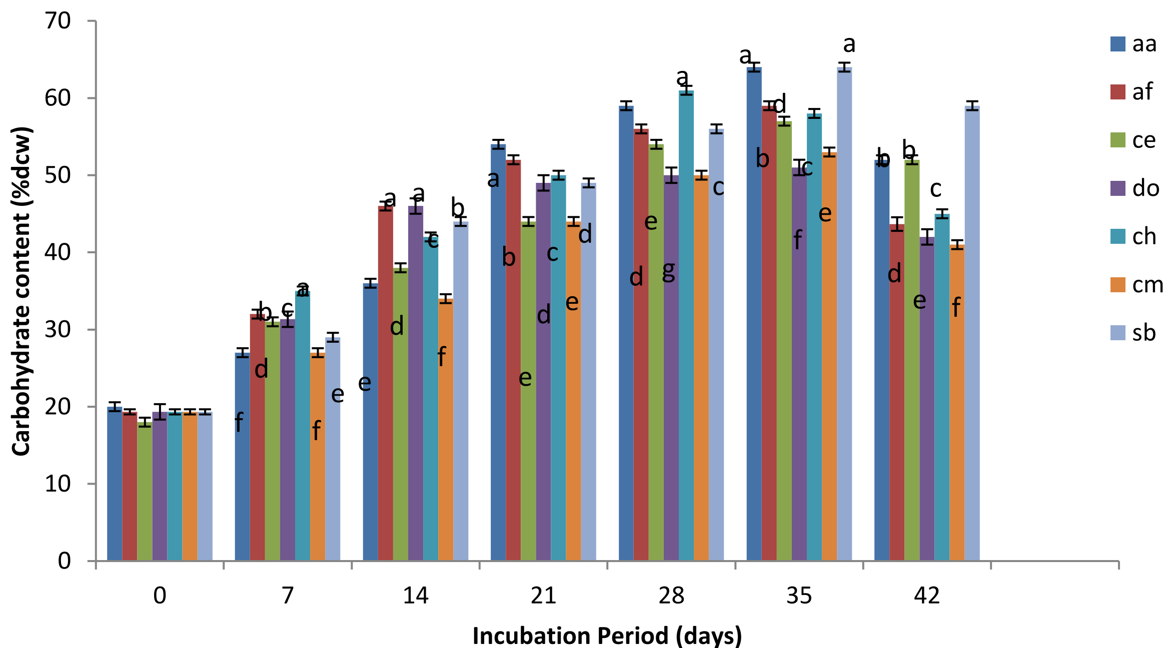

Fig. 3.3: Carbohydrate content of seven species in BG11 medium. Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test (Zar et al. (1984)) [43].

3.4. Estimation of Protein

From Figure 3.4, we can observe that in all the seven-test species, the protein concentration increased steadily till the 35th day. *Ankistrodesmus angustus* (aa) contain the highest protein concentration of 14.4% dcw among seven species while *Desmodesmus opoliensis* contains the lowest (5.96% dcw) among seven species. The gradual increase in the protein content of the test species may be due to the reduction of their chlorophyll content [7]. The details of protein content in all the species in stationary and log phase were given in Table III.

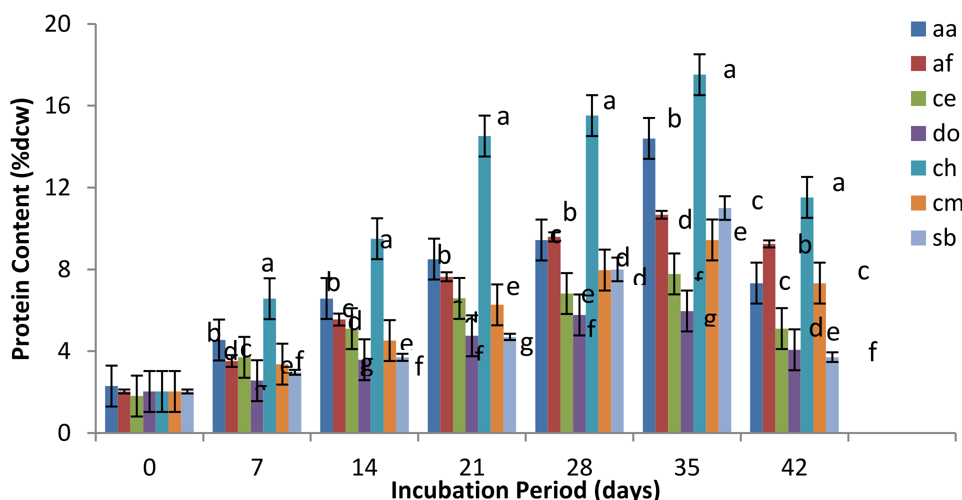

Fig.3.4: Protein content of seven species in BG11 medium. Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test (Zar et al. (1984) [43].

3.5. Estimation of lipid

The lipid accumulation in all the seven-test species under batch mode is presented in Figure 3.5 (A). Maximum accumulation of lipid was recorded in *Desmodesmus opoliensis* (31.11% dcw) among seven species while minimum in *Ankistrodesmus angustus* (12.66% dcw) among seven species on the stationary phase. The test microalgae have an appreciable amount

of lipid content and thus they are oleaginous in nature. Their lipid content range corroborates with that reported for other freshwater isolates under normal growth condition. From the graphical representation of the results, it is clear that the lipid accumulation was highest under stress condition, i.e., during the starved or stationary phase, when the cultures were grown in batch mode to achieve stationary phase. Acetyl-CoA Carboxylase (ACCase) is one of the key enzymes in the lipid synthesis pathway, which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, and it has been proved that it has a vital influence on lipid content [40]. It was reported in another algal species *Chlorella sorokiniana* that increased expression levels of ACCase might account for the increase in lipid content during the stationary phase of mixotrophic growth [35]. The details of lipid content in all the species in stationary and log phase were given in Table III.

Table III: Details of carbohydrate, protein and lipid content during log and stationary phase in BG11 media

Species Name	Carbohydrate Content (%dcw)		Protein Content (%dcw)		Lipid Content (%dcw)	
	Log Phase	Stationary Phase	Log Phase	Stationary Phase	Log Phase	Stationary Phase
A.angustus	54 ±0.5^{b}	64 ±0.5^{a}	8.5 ±0.4^{b}	14.4 ±0.5^{a}	9.485±0.38^{b}	12.66±0.5^{a}
A.falcatus	56 ±0.5^{b}	59±0.5^{a}	9.59±0.2^{b}	10.67±0.19^{a}	16.52±0.28^{b}	19.65± 0.2^{a}
C.ellipsoidea	44 ±0.5^{b}	57 ±0.5^{a}	6.58 ±0.5^{b}	7.78±0.29^{a}	21.69±0.4^{b}	28.18 ± 1^{a}
D.opliensis	49 ±1^{b}	51 ±1^{a}	4.75 ±0.2^{b}	5.96 ±0.16^{a}	25.59 ±0.25^{b}	31.11±0.48^{a}
C.humicola	50 ±0.5^{b}	61 ±0.5^{a}	14.52±0.28^{b}	17.52±0.33^{a}	14.52 ±0.3^{b}	21.33 ± 0.3^{a}
C.microporum	50 ±0.5^{b}	53 ±0.5^{a}	7.9 ±0.28^{b}	9.44 ±0.43^{a}	19.6 ±0.5^{b}	26.37 ±0.3^{a}
S.bijugatus	56 ±0.5^{b}	64 ±0.5^{a}	8 ±0.5^{b}	11 ±0.5^{a}	10.48 ±0.14 ^{b}	12.69±0.21^{a}

Note: Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test by Zar et al.1984 [43].

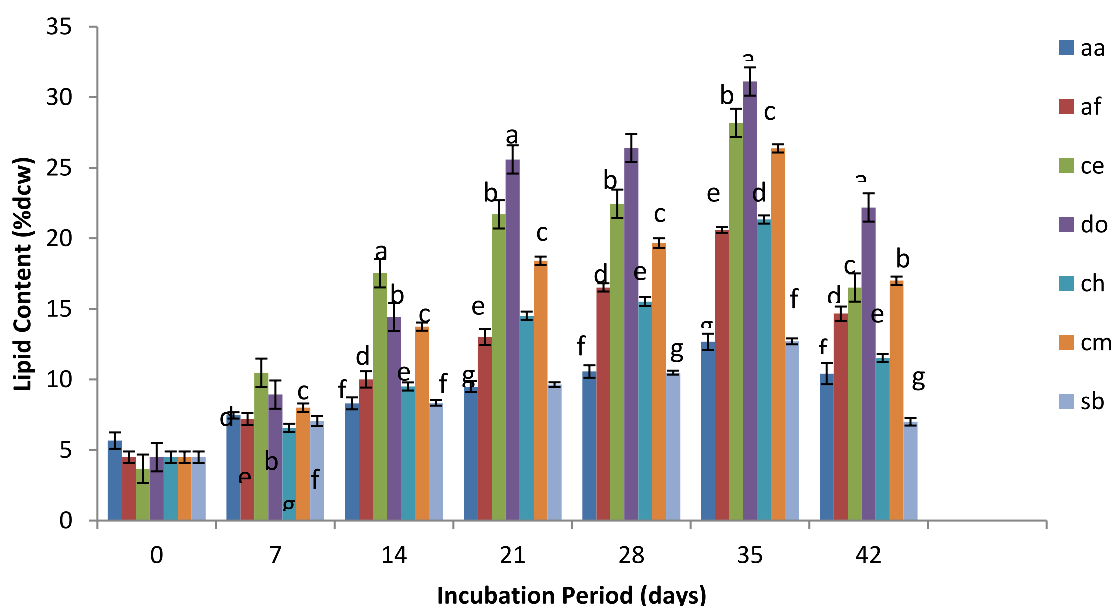

Fig. 3.5 (A): Lipid contents of seven species in BG11 medium. Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test (Zar et al. (1984)) [43].

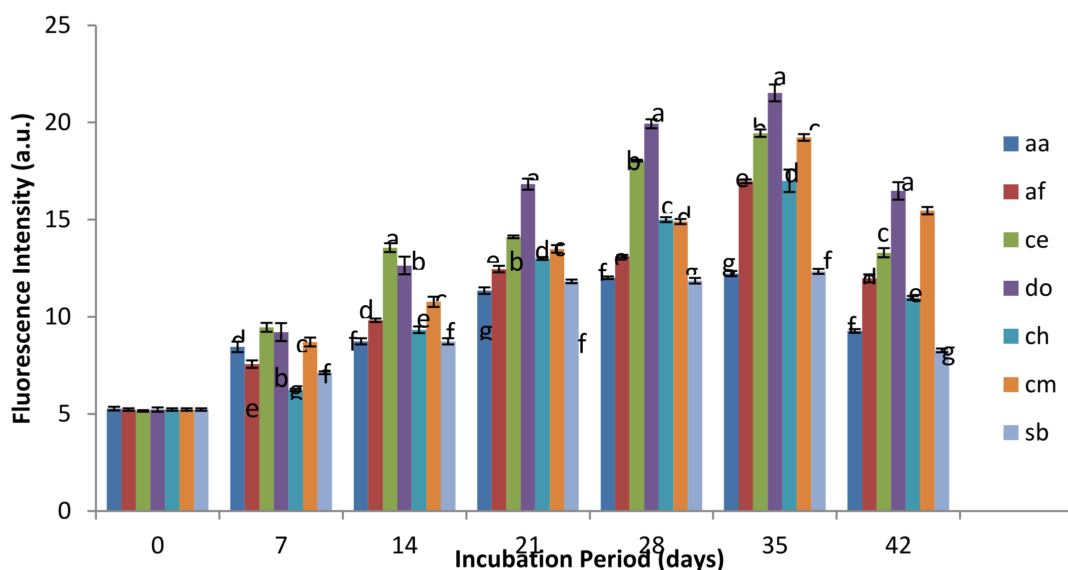

Fig.3.5 (B): Comparison of various culture fluorescence intensity with nile red dye. Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test (Zar et al. (1984)) [43].

Lipid accumulation was estimated by means of fluorescence spectrophotometer using Nile red dye. The results corroborated by that of the value estimated gravimetrically (Figs. 3.5(B)).

3.6. Composition of PUFAs

The seven algal species under study were found to have the capacity to accumulate higher amounts of lipid and are rich in polyunsaturated fatty acid (PUFAs) during stationary phase than log phase. During the log phase, Linoleic acid (C 18:2) was found to be present in all the seven species (Table IV), while during stationary phase other PUFAs *viz.* Docosahexaenoic acid (C 22:6), Hexadecatrienoic acid (C16:3), Hexadecatetraenoic acid (C16:4) Arachidonic acid (C 20:4) and Eicosadienoic acid (C 20:2) were detected (Table V).

Table IV: Fatty acids profile of seven species during log phase in BG11 media.

Fatty acids	**Relative % of FAMEs**						
SFA	***A.angustus***	***A.falcatus***	***C.ellipsoidea***	***D.opliensis***	***C.humicola***	***C.microporum***	**S.bijugatus**
C11:0		45±0.91^{a}		11±0.09^{e}			
C13:0			21±0.89^{b}	25.145±0.14^{b}		11.161±0.87^{d}	52.1±1.2^{a}
C16:0					13.213±0.12^{d}		
C17:0					11±0.1^{f}		
C18:0	41.01±0.089^{a}		32±0.089^{a}		10±0.09^{g}		
C19:0	12±0.098^{b}			13.32±0.08^{c}			
C20:0		32±0.56^{b}					
C22:0			21±0.143^{b}		12.234±0.089^{e}	44.79±0.65^{a}	
MUFA							
C16:1	10.34±0.6^{d}	6.5±0.47^{d}	2.786±0.6^{e}	29.21±0.56^{a}			
C18:1	7.45±0.7^{g}	4.5±0.32^{e}	8.755±0.7^{d}	11.224±0.07^{d}	13.573±0.078^{c}	32.149±0.45^{b}	36.877±0.4^{b}
C20:1	9.45±0.089^{e}				25.521±0.76^{a}		
C24:1	8.45±0.076^{f}						
PUFA							
C18:2	11.3±0.047^{c}	12 ±0.008^{c}	14.459±0.114^{c}	10.101±0.006^{f}	14.459±0.004^{b}	11.9±0.0043^{c}	11.023±0.009^{c}

Note: Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test by Zar et al.1984 [43].

Table V: Fatty acids profile of seven species during stationary phase in BG11media. Bars indicate mean ± SE of four replicates.

Fatty acids	Relative % of FAMEs						
SFA	***A.angustus***	***A.falcatus***	***C.ellipsoidea***	***D.opliensis***	***C.humicola***	***C.microporum***	**S.bijugatus**
C11:0		33±0.91^{a}		11±0.09^{e}			
C13:0			16±0.89^{d}	19.729±0.14^{b}		11.161±0.87^{e}	52.075±1.2^{a}
C16:0					7.213±0.12^{d}		
C17:0					11±0.1^{e}		
C18:0	41.01±0.089^{a}		27±0.089^{a}		4±0.09^{h}		
C19:0	12±0.098^{c}			13.32±0.08^{c}			
C20:0		32±0.56^{b}					
C22:0			16±0.143^{d}		6.234±0.089^{g}	39.175±0.65^{a}	
MUFA							
C16:1	10.34±0.6^{d}	6.5±0.47^{e}	2.417±0.6^{f}	23.794±0.56^{a}			
C18:1	6.35±0.7^{g}	4.5±0.32^{f}	3.755±0.7^{e}	11.224±0.07^{d}	13.573±0.078^{c}	26.534±0.45^{b}	36.877±0.4^{b}
C20:1	9.45±0.089^{e}				19.38±0.76^{a}		
C24:1	8.45±0.076^{f}						
PUFA							
C16:3			16.228±0.047^{c}				
C16:4						11.33±0.015^{d}	
C18:2	12.4±0.03^{b}	13.19±0.002^{c}	18.6±0.04^{b}	10.107±0.004^{g}	15.6±0.003^{b}	11.8±0.002^{c}	11.048±0.008^{c}
C18:4					12.09±0.014^{d}		
C20:2				10.826±0.019^{f}			
C20:4		10.810±0.02^{d}					
C22:6					10.91±0.0003^{f}		

Note:Bars indicate mean ± SE of four replicates. Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test by Zar et al.1984 [43].

Table VI: Comparrisson of fatty acids profile of seven species during log and stationary phase in BG11media.

Species Name	Relative % of FAMEs					
	Total SFA		**Total MUFA**		**Total PUFA**	
	Log Phase	**Stationary Phase**	**Log Phase**	**Stationary Phase**	**Log Phase**	**Stationary Phase**
A.angustus	53.01^{a}	53.01^{a}	35.69^{a}	34.59^{b}	11.3^{b}	12.4^{a}
A.falcatus	77^{a}	65^{b}	11^{a}	11^{a}	12^{b}	24^{a}
C.ellipsoidea	74^{a}	59^{b}	11.541^{a}	6.172^{b}	14.459^{b}	34.828^{a}
D.opliensis	49.5^{a}	44^{b}	40.4^{a}	35^{b}	10.1^{b}	20.9^{a}
C.humicola	46.4^{a}	28.4^{b}	39^{a}	32.9^{b}	14.5^{b}	38.6^{a}
C.microporum	55.9^{a}	50.3^{b}	32.1^{a}	26.5^{b}	11.9^{b}	23.1^{a}
S.bijugatus	52.1^{a}	52^{b}	36.9^{a}	36.9^{a}	11.023^{b}	11.048^{a}

Note: Different superscripts indicate significant differences ($p < 0.05$) by Duncan's multiple range test by Zar et al.1984 [43].

Linoleic acid is an essential fatty acid, belonging to an omega-6-fatty acid group, which means it cannot be produced by the human body and has to be acquired through diet. It is a precursor for the synthesis of very long chain fatty acids in the human body. It is mostly present in plant oils, fish and microalgae [15,28,41]. In this study, Linoleic acid is found to be present in all the freshwater green microalgae, when grown in BG11 medium during log phase under special growth condition established in the laboratory (unpublished) which make this group of microalgae as a potential dietary source of essential fatty acid.

Docosahexaenoic acid (C 22:6) was found to be present in *Chlorococcum humicola* besides Stearidonic acid (C 18:4). Docosahexaenoic acid is one of the most important and sought- after omega-3-fatty acid, which is known to be essential for human brain development in infants. Its role in

the prevention of Alzheimer's disease is under investigation [8]. This fatty acid is reported to be mostly present in marine fishes, shellfish and some marine algae [25,26]. To the best of our knowledge, this is the first report of assimilation of Docosahexaenoic acid in *Chlorococcum humicola* under nutrient starved condition and special growth condition established in the laboratory (unpublished). The presence of this important fatty acid may be attributed to the presence of Stearidonic acid, a short chain omega-3-fatty acid, as it is said to be a precursor for long chain fatty acids like Docosahexaenoic acid and Ecosapentaenoic acid. Besides, it does not require desaturase enzyme for conversion to a long chain fatty acid. It is generally present in low amount in seed oil, sardines and marine algae [34, 42]. Consumption of Stearidonic acid is reported to enhance the Ecosapentaenoic acid level by five-fold in plasma, heart, neutrophil and erythrocyte phospholipids [42]. Though Stearidonic acid is a lesser- known omega-3-fatty acid, its role in the human diet is gradually increasing in recent times.

Another two omega-3-fatty acids *viz.* Hexadecatrienoic acid (C16:3) and Hexadecatetraenoic acid (C16:4) was found in *C. ellipsoidea and C. microporum* respectively. The specific role of these fatty acids is not known but has health benefits as reported for PUFAs.

Besides, omega-3-fatty acids, two omega-6-fatty acids were detected *viz.* Arachidonic acid (C 20:4) in *Ankistrodesmus falcatus* and Eicosadienoic acid (C 20:2) in *Desmodesmus opliensis*. Their roles in human physiology are primarily mediated through their conversion to n-6 eicosanoids which binds to various receptors in the body [19].

Arachidonic acid is an integral constituent of the cell membranes, which provides selective permeability, flexibility, and fluidity of the membranes [4]. It is one of the major PUFA of brain cell phospholipids [17]. It helps in maintaining the fluidity of the cell membranes of the hippocampal cells and is thus essential for the normal neural function [11]. It is generally present in animal meat, seafood, fish and eggs due to which the only source for vegetarians is linoleic acid, an essential fatty acid, from which the body can synthesize Arachidonic acid. In this investigation, we detected this fatty acid in freshwater microalgae when grown in a unique cultural condition established in the laboratory in the particular growth phase while it is mostly reported from the marine ones.

As regards to Eicosadienoic acid, it is a naturally occurring PUFA, which is predominantly found in human milk. Since this fatty acid is detected in the freshwater microalgae *Desmodesmus opliensis*, this alga may be a dietary source for this fatty acid.

4. CONCLUSION

From this study, we found that all the seven species of freshwater green microalgae contains linoleic acid, an essential fatty acid, on culturing in BG11 medium containing Sodium nitrate and maintained under unique cultural conditions established in the laboratory (unpublished) which makes them suitable for use as a dietary source of PUFA since very long chain fatty acids can be synthesized from it by the human body. Microalgal species *viz. Ankistrodesmus falcatus, Chlorella ellipsoidea*, *Desmodesmus opoliensis, Chlorococcum humicola* and *Coelastrum microporum* produced other important PUFAs, as well, including Docosahexaenoic acid during the stationary phase of their growth cycle. Thus, these green microalgae in stationary phase hold promise for use as nutraceuticsals if they grow in BG11 medium containing sodium nitrate under unique culture condition established in the laboratory in batch mode. This studies solely established unique culture condition and determine the particular stage of the growth phase, which causes enhancement of PUFAs content of freshwater microalgae.

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***Corresponding Author:**
Ajitabh Bora*
Email: **ajitabhbora@gmail.com**