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Growth, Total Lipid content and Fatty Acid Profile of a Native Strain of the Freshwater Oleaginous Microalgae *Ankistrodesmus falcatus* (Ralf) grown under Salt Stress Condition

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Abstract

Growth, fatty acid compositions and calorific value of a native freshwater oleaginous microalgae A.falcatus was studied in batch culture at light intensity 40 µ mol photons/m^2/s, temperature 25 ± 2 ^{0}C and 16:8 h light and dark diurnal cycles. Improved growth and total lipid contents were determined with the culture grown under salinity up to 160 mM. The highest specific growth (μ=0.313 d^{-1}) and least doubling time (T_2=2.21 days) with maximum increase in cell numbers (29.0 x 10^5 ml^{-1}) were recorded in medium supplemented with 160 mM of NaCl compared to control medium (μ=0.209 d^{-1}, T_2=3.32 days and 15.2 x 10^5 ml^{-1} respectively). Improved total lipid (55.3%), carbohydrate (14.5%) and protein (4.8%) contents were also determined in the culture under salinity compared to control medium (lipid 38.3%, carbohydrate 12.6% and protein 3.1% respectively). C16:0, C18:1 and C18:3 were found to be the major fatty acid components of the lipid content. Marginal increase in C18:1 (30.5%) was observed in culture grown under salinity stress. With maximum energy value of 27.9 ± 0.15 kJg^{-1}, a close correlation (R^2 = 0.949) between lipid content and calorific value was observed. With further augmentations of lipid content and improved fatty acids, the native microalga strain could be a potent candidate for biofuel production.

Keywords: *Ankistrodesmus falcatus,* biofuel, fatty acid methyl ester, microalgae, salinity.

Introduction

Energy from renewable biomass sources has gained global attention with the potential to substitute for fossil fuels in all energy markets – in the production of heat, electricity, and fuels for transport. Concerted efforts are now underway globally to harness alternative energy from renewable biomass sources generated *via* photosynthesis to move towards more sustainable bio-based economies, where biomass-derived products substitute for petrochemical-derived products[1]. Development of first and second generations of biofuels, which are based on different categories of biomass feedstock and their downstream processing and typically obtained from starch, vegetable oil, and cellulosic crops, require cultivated land and water supplies that can compete with land use for food production[2-4]. Extensive cropping of plants for biofuel production, which will certainly take place with the increasing fuel demand, raises a food *vs* fuel dilemma, and also natural resource demand problems[5-8]. With such limitations of first and second generation biofuels, microalgae based third and further generations of biofuel have gained great interest[9-14].

Microalgae are considered to represent the most promising among existing renewable biomass sources to generate biofuels. They are also likely to have much lower adverse effect on the environment and on the world's food supply than conventional biofuel-producing crops. The unique diversity of microalgae and the spectrum of species available for amenability for biofuel production as compared to other advanced biomass feedstock have placed microalgae on the priority list. Various species may be selected to optimize the production of different biofuels. Microalgae biomass can be utilized to produce varieties of fuels, such as liquid fuels and gas, gas-or oil-based biofuels, bioethanol or methanol, biohydrogen and biodiesel[2, 3, 5, 10, 11, 12, 15-24]. Moreover, microalgae offer a diverse spectrum of valuable products and pollution solutions, such as food, nutritional compounds, omega-3 fatty acids, animal feed, organic fertilizers, biodegradable plastics, recombinant proteins, pigments, medicines, pharmaceuticals, and vaccines[25].

To enhance the economic feasibility of algal oil production, biomass productivity (production per unit volume per unit time), cellular lipid content, and overall lipid productivity are the three key parameters that need to be improved. These requirements are not always compatible, and in general, conditions favoring a high growth rate of cells result in a low lipid fraction in the cells and vice versa[26]. High lipid content with favorable fatty acid compositions[27], faster growth rate, ease of harvest, and dominance in nature with the ability to adapt in the prevalent climatic conditions are some of the desirable criteria for choosing the right species for biofuel production[23]. Isolation of native strains from local water bodies can provide the best information regarding the selection of appropriate strain(s) in terms of biomass and lipid productivity, dominance in the native

algal flora, and hence the likelihood of being the most suitable selection as a biomass feedstock[28-30]. In addition to the species selection, nutritional requirement for culture optimization is another essential factor that significantly affects the growth rate and yield of products[31]. Some microalgae species can double its biomass in less than 24 h under optimum culture conditions[10]. Such high yield and high density biomass is ideal for intensive cultivation and can provide an excellent biomass source of biofuels. Oil levels 20 to 50% by dry weight biomass are quite common, and some can exceed 80%[32]. Significant increases in lipid contents have been reported in microalgae after being subjected to various stress conditions during cultivation such as nitrogen deprivation, high light intensity, high salinity, phosphate limitation or co-immobilized in alginate beads with the bacterium *Azospirilium brasilense*[11,18,33-37]. However, stress conditions also have negative influence on growth, resulting low overall biomass production. Therefore, it is more appropriate to apply stress conditions in the later stage of growth after initial biomass production[38].

Besides high lipid content, another important characteristic for any biodiesel feedstock is the suitability of the fatty acid profile for biodiesel production. Oils and fats are primarily composed of triacylglycerols (TAGs), which serve as a primary storage form of carbon and energy in microalgae; their fatty acid composition is also superior to that of other cellular lipids (phospholipids and glycolipids) for biodiesel production. Fatty acid compositions of a particular lipid influence the saponification number, iodine value of the lipids, and biodiesel quality, such as cetane number, heat of combustion, oxidative stability, cloud point, and lubricity[39,40]. Algal fatty acid composition is influenced by growth conditions such as temperature and nutrient availability[41]. The different carbon chain lengths and number and position of unsaturated bonds found in fatty acids influence the cetane number (CN), iodine value, oxidative stability, cold flow properties and viscosity of the fuel[42].

Production of liquid biofuels, such as biodiesel from microalgae involves to some extant or more extraction or conversion before the algae can be used as a biofuel. The direct uses of microalgae biomass do not involve such processing and thus minimize the cost. The use of dried powder of the microalgae *Chlorella* as fuel together with a mixture of 85% cellulose powder and 15% diesel to run a diesel engine was reported[18]. A high calorific value of the microalgae is essential to be act as a fuel, which chiefly depends on the lipid content besides carbohydrate and protein. An increasing calorific value is directly related to an increase in lipid content[18].

The present study was focused on influences of salinity on growth and lipid yield of a native Indian strain of the freshwater microalgae *A.falcatus* towards potential biomass utilization. The principal objectives were to investigate the influences of medium compositions and salinity on growth, lipid yield and calorific value of the biomass.

Material and Methods

Microalgae Strain Isolation: Freshwater microalgae *A. falcatus* was isolated from water samples collected from the wetland Deepor Beel (latitude 26^0 13´ N and longitude 91° 66´ E), Guwahati, Assam, India. Isolation of the microalgae and its mono-culture was raised in BG11 medium after several dilutions and streaking into agar plate according to following standard procedure[43, 44]. Purity of the culture was periodically checked using an optical microscope (LOBAMED, Model: ATC 2000) as well as streaking into agar plates. Subculture was done after every third week of inoculation.

The isolated strain was studied under optical microscope for morphological identification. Microphotographs were taken with the help of a digital camera (SONY Model: DSC-W100; 8.1 MP).

Growth medium and Culture conditions: Monocultures were raised and maintained aseptically in autoclaved BG11 medium under laboratory conditions. The various media compositions used in this investigation are depicted in table - 1. Culture pH was adjusted to 7.5 with either 1N HCl or 1N KOH prior to autoclaving. Growth conditions were: irradiance 40 μ mol photons $m^{-2}s^{-1}$, temperature 25 ± 2 ^{0}C and photoperiod of 16 h light: 8 h dark diurnal cycles.

Table - 1
Culture medium composition

Constituents (g L^{-1})	BG11
$NaNO_3$	1.5
$CaCl_2$ $2H_2O$	0.036
$MgSO_4 7H_2O$	0.075
K_2HPO_4	0.4
EDTA	0.001
Citric acid	0.006
Na_2CO_3	0.2
$NaMoO_4$	2.1 x 10^{-5}
$ZnSO_4 7H_2O$	2.2 x 10^{-4}
$MnCl_2 4H_2O$	1.8 x 10^{-3}
$CuSO_4 5H_2O$	0.8 x 10^{-5}
H_3BO_4	2.8 x 10^{-3}

Influences of Salinity on Growth and Total Lipid Content: Salinity induced influences on growth responses of the microalgae strain were studied in BG11 medium supplemented with NaCl in increasing order (40, 80, 160 and 320 mM). BG11 medium without addition of NaCl was used as control. The study was conducted in batch mode using 100 ml of medium in 250 ml volume Erlenmeyer flasks. Exponentially growing culture (20% v/v) was used as inoculums. Studies were conducted in triplicate under similar growth conditions as stated earlier. Cells harvested after 10 days of culture durations were compared in terms of total lipid content in each of the treatments.

Growth Evaluation and Biomass Determination: Growth characteristics were determined from the respective growth curves developed by plotting the observed cell numbers (cells/ml) against time of observations (days). Cell numbers were determined from direct cell counts using a Neubour Haemocytometer. The specific growth rate (μ) and doubling time (T_2) were calculated using equations (1) and (2)[45]:

$$\mu = \ln (N_2/N_1) / (t_2-t_1) \quad (1)$$

$$T_2 = 0.6931/\mu \quad (2)$$

Where, N_1 and N_2 are the biomass at time (t_1) and time (t_2), respectively.

Biomass was determined as dry cell weight (DCW) and measured gravimetrically. A known volume of culture was filtered through pre-weighed and pre-combusted GF/C filter paper. The filtered cell mass was oven dried at 80°C for 6 hrs until constant weight, cooled down to room temperature in desiccators and measured the dry weight of the sample using an analytical balance with a precision of 0.1 mg. Biomass was expressed in grams per liter (g L^{-1}).

Determination of Total Cellular Contents: Total cellular protein and carbohydrate contents were determined from a known amount of freeze dried cell biomass harvested at the end of the logarithmic phase of growth following standard procedures[46,47].

Total lipid content was determined quantitatively. A known volume of culture was harvested during the late exponential phase of growth by centrifugation at 2000 *g* for 2 minutes, rinsed with 1% NaCl solution, followed by rinsing with double-distilled water twice. The biomass was lyophilized using a freeze-dryer (ALPHA 1- 4, Germany) for 36-48 hrs and kept in desiccators until constant weight. Total lipid was extracted from 1 g of the lyophilized cell biomass using a solvent mixture of chloroform and methanol (2:1)[48]. The residue was re-extracted (2 to 3 times) with chloroform. The filtrate was taken in a separatory funnel, and lipids were obtained in the bottom chloroform layer formed upon addition of 0.9% NaCl and collected in a grease-free cleaned pre-weighted glass vial. The solvent was removed using a rotary vacuum evaporator (JSGW, India) at 50 ºC to near dryness and dried in desiccators under reduced pressure over anhydrous silica gel (ACS, Sigma-Aldrich, USA). The dried lipid extract was measured gravimetrically and expressed as percent total lipid dry cell weight (% DCW).

Analysis of Fatty Acid Methyl Ester (FAME): Approximately 20 mg of crude lipid extract was used to prepare the FAME. Transestrification of the lipid sample was performed using a reaction mixture of methanol: acetyl chloride (20:1 v/v) [49]. Crude lipid (20 mg) was taken in Teflon lining glass vial and added 2 ml *n*-hexane containing internal standard methyl C-13 (2 mg ml^{-1}). Freshly prepared above stated methylation reaction mixture (5 ml) was then added to the mixture. The vial was filled with nitrogen gas and mixed well by vortexing at low speed followed by incubation at 75 ^{o}C for 60 min. The mixture was allowed to cooled down to room temperature (30 ± 2 ^{0}C), then added 5 ml of double-distilled water containing 2.5% K_2CO_3 and 2 ml of chloroform: *n*-Hexane (1:4 v/v) followed by brief vortexing for about 30 to 40 seconds. The mixture was centrifuged at 1957 *g* for 1 min and the upper hexane layer containing FAME was collected in a clean glass vial. The extracted FAME was dried by passing through anhydrous Na_2SO_4 and analyzed using a gas chromatograph (DANI GC1000, Italy) equipped with a flame-ionized detector and DN WAX capillary column (30 m x 0.25 mm x 0.25 m). The column temperature was increased from 150 ^{o}C to 230 ^{o}C at a rate of 3.5 ^{o}C min^{-1}. Fatty acid components were identified by comparing the retention time against known standard.

Determination of Calorific Value (CV): Biomass energy value in terms of calorific value (CV) was determined using an automatic adiabatic bomb calorimeter (Model: RBC 106/09) according to DIN 51900 T3 (testing of solid and liquid fuels, determination of gross calorific value by the bomb calorimeter and calculation of net calorific value; method with adiabatic jacket)[18]. A crucible containing moisture-free dry algal biomass (1 g) was ignited in presence of oxygen (99.99% purity) at 3000 kPa. Calorific value was calculated from the resulting increase of water temperature using equation (3).

$$CV = (WE \times \Delta T/WM) \times (4.18400 \times 10^{-3}) \text{ kJ g}^{-1} \quad (3)$$

Where, W_E enthalpy of water equals to 2568.293 cal g, ΔT differences in temperature, W_M sample weight

Results and Discussion

Microscopic observations revealed that the cells are fusiformis, solitary, or clustered of individual cells without colonial sheath; chloroplast parietal, pyrenoids absent, variable in size within the same plant mass; cells are 30 to 36 μm long and 2.5 to 4.5 μm in diameter (figure - 1). Based on the morphological data the newly isolated native strain of the freshwater microalga was initially identified as *Ankistrodesmus falcatus* (Corda) Ralfs, (Class: Chlorophyceae; Family: Selenastraceae).

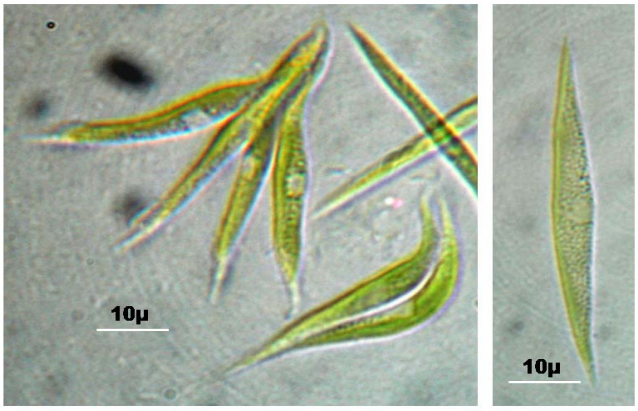

Figure - 1
***A.falcatus* cells under optical microscope (magnification 400x)**

Influence of Salinity on Growth and Total Lipid Content: Influence of salinity (NaCl concentration) on growth and lipid content of the tested *A.falcatus* strain was observed with the growth behavior, grown in BG11 medium supplemented with NaCl in increasing order. The strain was found to grow in all the five concentrations of NaCl (40 to 320 mM) (figure - 2). Best result was obtained in medium supplemented with 160 mM followed by 80 and 40 mM of NaCl. Growth rate was however retarded in the culture medium containing 320 mM of NaCl. Culture medium containing 160 mM of NaCl also revealed a net increase in cell numbers (29 x 10^5 ml^{-1}) compared to that of control medium (15.2 x10^5 ml^{-1}) at the terminal day (day 10) of the experiment (table 2). The highest specific growth rate (0.313 d^{-1}) and least doubling time (2.21 days) were observed in the medium supplemented with 160 mM of NaCl compared to control medium (0.209 d^{-1} and 3.32 days respectively). However, further increase in salt concentration beyond 320 mM retarded the growth of the studied microalgae strain. Similar finding on enhanced growth at low salinity level was also reported in *Chlorella vulgaris* and *Chlorococcum humicola*[50]. Significant increase in growth rate of *C.vulgaris* was reported at low salinity level of 50 to 100 mM. Further increase in salinity beyond 200 mM retarded the growth in both *C.vulgaris* and *C.humicola*. Similarly, increase in biomass production was reported in *B.braunii* at low salinity level of 17 to 34 mM[34]. However, the growth rate was significantly reduced at higher concentration of NaCl. Based on the salt tolerance extent algae were grouped as halophilic (salt is required for optimum growth) and halotolerant (having response mechanism to survive in saline condition). In both cases, the algae produce some secondary metabolites in order to cope up with salinity induced changes in growth conditions and also balance as per the surrounding osmotic[51].

Total lipid, carbohydrate and protein contents were also found to be improved in medium containing NaCl up to 160 mM (table 2). Salinity induced enhanced lipid and other biochemical contents (such as keto carotenoid astaxanthin) were reported in a number of microalgae species[34, 52-54]. The studied *A.falcatus* strain under a salinity stress revealed improved total lipid content. The highest lipid content (55.3% DCW) was recorded in the cultures containing 160 mM of NaCl, which was nearly ≈1.4 fold increase in total lipid content than the total lipid content (38.3% DCW) of cultures grown in control medium. However, slightly reduced amount of total lipid content (52.5% DCW) was recorded in culture grown at the highest salinity level (320 mM), which might be resulted due to the production of less biomass in medium with highest salinity. Similarly, carbohydrate and protein content were also found to be increased with the increasing salinity. The highest carbohydrate (14.5% DCW) and protein (4.8% DCW) contents were recorded in the medium containing 160 mM of NaCl and the least (carbohydrate 12.6% and protein 3.1%) in control medium. Similar observation was also reported[34] in the hydrocarbon rich *B.braunii* culture.

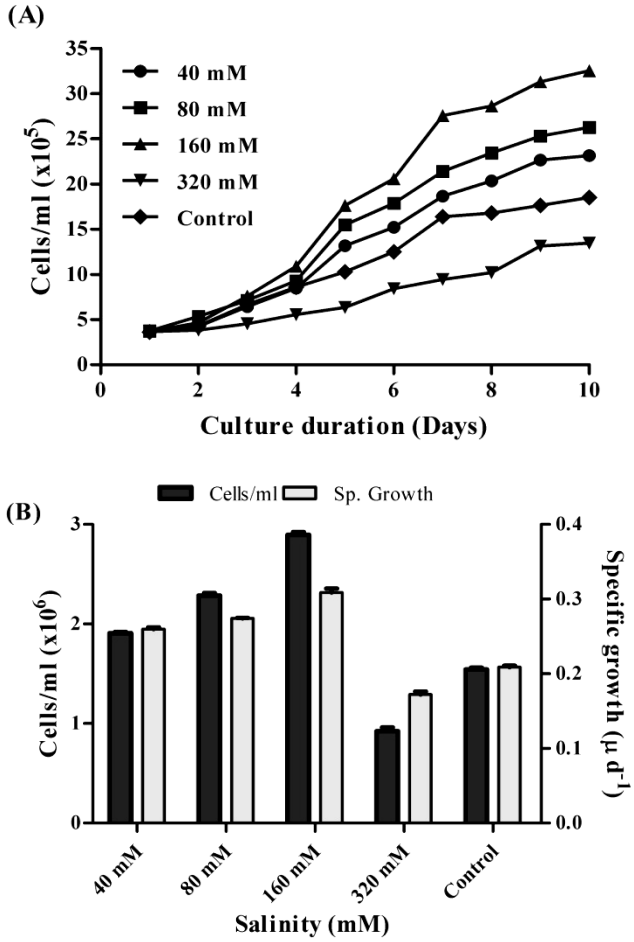

Figure - 2 (A-B)
Influence of salinity on growth of *A.falcatus*. (A) Growth characteristics under increasing order of salinity; (B) Increased in cell numbers and specific growth under various salt concentrations. Error bars represent standard error (n=3)

Fatty Acid Methyl Ester (FAME) profile: FAME compositions of *A.falcatus* biomass grown in control BG11 medium and under salinity-stressed (BG11 + 160 mM NaCl) conditions were evaluated (table 3). FAME analysis revealed nearly similar fatty acid profile in the both culture conditions. Palmitic (C16:0), oleic (C18:1), linolenic (C18:2), and linolenic (C18:3) acids were found to be the major components of the oil derived from *A.falcatus* strain. The percentage of saturated fatty acids (SFA = 36.2%) was higher in culture grown under normal BG11 medium than the SFA (32.1%) of stress culture. Both monounsaturated fatty acid (MUFA = 31.1%) and polyunsaturated fatty acid (PUFA = 27.9%) were significantly higher in culture grown under NaCl stress condition. The percentage trienoic fatty acids (TFAs) were similar to that of C18:3 as other TFAs were not detected. Among the SFAs, C16:0 was the most dominant fatty acid of the studied *A.falcatus* strain in both normal and stress conditions (28.6%

and 26.9% respectively). Oleic acid (30.5%) was the most dominant fatty acid of MUFAs, which increased significantly in culture grown under stress condition. Although the amount of C18:3 (15.7%) was slightly reduced in culture grown under salt stress condition than normal culture condition (16.7%), overall PUFA content was higher under stress condition. The degree of unsaturation (DU) calculated from individual fatty acid contents was also found higher in the studied *A.falcatus* strain grown under salinity stress (86.9%) than the DU of control medium (79.5%). The percentage of very long chain fatty acids (VLCFAs) was higher in the studied *A.falcatus* biomass grown in control medium (4.3%) compared to the VLCFAs of biomass grown under salinity stress (2.3%).

Irrespective of the lipid types found in the different taxa of freshwater Chlorophyceae, fatty acids C16:0, C18:1, C18:2 and C18:3 were reported as the common fatty acid types[55]. In the present study, the FAME analysis of the native *A.falcatus* strain revealed C16:0, C18:1 and C18:3 were the major fatty acid components in the both control and salinity stress conditions of which, C16:0 and C18:1 contributed 28.6% and 26.8% relative to the control. The results were in accordance with the results from previous studies in *Chlorella zofingiensis* grown in presence of glucose[56, 57]. Significant increase in C18:1 (30.5%) was observed under salinity stress condition. Similar observation was also reported in the marine microalgae *Dunaliella salina*[58]. Among the PUFAs, slightly higher content (16.7%) of α - linolenic (C18:3) acid was observed in the studied *A.falcatus* strain grown in control medium than in NaCl stress condition (15.7%). Otherwise, the content of C18:2 (12.2%) was significantly increased under salinity stress than under control medium (9.2%). Palmitic, linoleic, and α - linolenic acids were reported as the major fatty acids in *Ankistrodesmus* sp, *C. vulgaris* and *Chlamydomonas reinhardtii*[59, 60].

Table - 2
Growth Characteristic, Total Lipid Content and Calorific Value of *A.falcatus* strain under Salt Stress Conditions

Salinity (mM NaCl)	Specific growth (μd^{-1})	Doubling time (T_2 days)	Cell numbers ($x10^5$ ml^{-1})	Lipid (% DCW) [a]	Calorific value ($kJgm^{-1}$) [b]
Control	0.209	3.32	15.0	38.3 ± 0.20	19.1 ± 0.05
40	0.263	2.64	19.3	38.6 ± 0.12	19.7 ± 0.11
80	0.275	2.52	22.8	43.0 ± 0.15	23.6 ± 0.05
160	0.313	2.21	29.0	55.3 ± 0.01	27.9 ± 0.15
320	0.179	3.87	9.7	52.4 ± 0.22	26.7 ± 0.05

[a] values are mean of three independent values; [b] values are mean of five independent values, '±' represents standard error of means

Table - 3
Fatty Acid Methyl Esters (FAME) Compositions (% wt) of *A.falcatus* strain under Salt Stress and Normal Conditions

Fatty acid	Salinity (mM)	
	0.0 mM	160 mM
Palmitic acid (16:0)	28.6	26.9
Stearic acid (18:0)	4.2	3.1
Oleic acid (18:1)	26.8	30.5
Linoleic acid (18:2)	9.2	12.2
Linolenic acic (18:3)	16.7	15.7
Behenic acid (22:0)	0.5	0.8
Erucic acid (22:1)	0.9	0.6
Lignoceric acid (24:0)	2.9	1.3
Unidentified	10.2	8.9
$\sum$ SFA [a]	36.2	32.1
$\sum$ MUFA [b]	27.7	31.1
$\sum$ PUFA [c]	25.9	27.9
$\sum$ TFAs [d]	16.7	15.7
$\sum$ VLCFAs [e]	4.3	2.7
DU [f]	79.5	86.9

[a] *$\sum$ SFA (%wt) = $\sum$ Cn:0,* [b] *$\sum$ MUFA (%wt) = $\sum$ Cn:1,* [c] *$\sum$ PUFA (%wt) = $\sum$ Cn:2 + $\sum$ Cn:3,* [d] *$\sum$ TFA (%wt) = $\sum$ Cn:3,* [e] *$\sum$ VLCFAs (%wt) = $\sum$ C20:n + $\sum$ C22:n + $\sum$ C24:n,* [f] *DU (%wt) = $\sum$ Cn:1 + 2 x ($\sum$ Cn:2 + $\sum$ Cn:3)*

Calorific Value (CV): The calorific value of the studied *A.falcatus* biomass was found to vary with total lipid content that influenced by increasing salinity (table - 2). The calorific value was improved with the increasing salinity up to 160 mM, which directly influenced the improved lipid content of the biomass (figure - 3A). The highest calorific value (27.9 kJ g^{-1}) was obtained with biomass produced from culture grown under 160 mM of NaCl. The microalgae *A.falcatus* grown in control BG11 medium containing a relatively less total lipid (38.3%) content revealed comparatively lesser CV (19.1 kJ g^{-1}) (table - 2), which was in close agreement with earlier report[18]. The principal contribution to the CV of cell biomass is from their total cellular carbohydrate, protein and lipid content[18, 61, 62]. Microalgae grown under normal conditions were reported to have CVs between 18 to 21 kJ g^{-1}, which is much lower than the CV of mineral diesel (42 kJ g^{-1})[18]. However, an improved calorific value was reported by improving the total lipid content of the biomass[18,61], and also evident from the results of our present study (table - 2, figure - 3). The tested *A.falcatus* strain grown in BG11 medium containing elevated level of NaCl yielded an increased level of total lipid content revealed increasing CVs (table - 2). Similar results were also reported by in *C.vulgaris* and *C.emersonii*[18]. A linear trend of increasing CV with increasing total lipid content of the studied microalgae *A.falcatus* strain was observed with a value of $R^2 = 0.955$ (figure - 3B).

It is envisaged that microalgae biomass would be used as renewable biofuel for electricity generation using static diesel engines for which large microalgae biomass production with high productivity and high lipid content must need to be maintained[18, 62]. The native *A.falcatus* strain achieved a better growth and thereby higher biomass yield in BG11 medium supplemented with 160 mM NaCl compared to control medium (0.0 mM NaCl). The lipid content reached up to 55.3% with an increasing CV (27.9 kJ g^{-1}), under the salt stressed condition. As seen from figure - 3B, a linear increase ($R^2 = 0.949$) of CV with increasing lipid content was observed, which signify that the intercellular lipid content together with carbohydrate and protein largely influence the resultant CV of the tested *A.falcatus* cell biomass. The results were in accordance with previous reports [18, 61-63].

Conclusion

Salinity induced improved growth rate, total lipid content, and energy value in terms of calorific value was recorded in the studied native strain of oleaginous freshwater microalgae *A.falcatus* biomass. Salinity-induced improved growth and lipid content were evident from the salinity tests using BG11 with increasing concentration of NaCl.

∑ SFA (32.1%) was decreased under salinity stress, while ∑ MUFA (31.1%) was increased. Content of C18:1 (30.5%) was improved under salinity than that of control (26.8%). ∑ TFA (15.7%) content was decreased slightly in the FAME contents of lipid fraction under salinity stress, however, ∑ PUFA (27.9%) content was marginally increased compared to that of control (25.9%). The ∑ VLCFA (2.7%) content was decreased considerably. The DU (86.9%) was increased considerably under salinity stress in comparison to control (79.5%). The CV of the studied *A.falcatus* strain biomass grown under normal BG11 medium revealed 19.1 kJ g^{-1}, which was much lower than that of petroleum diesel (42 kJ g^{-1}). CV was improved up to 27.9 kJ g^{-1} in biomass produced under salinity stress. Further culture optimization for increased biomass yield with concomitant lipid content and improved of fatty acid profile, the native *A.falcatus* could be a potent biomass feedstock towards biofuel production. With the support from present research findings the native strain of freshwater oleaginous microalgae *A.falcatus* was found to be potential renewable biomass source towards production biofuel, although further studies are needed for optimization of mass cultivation of the strain for utilization as renewable biomass feedstock of biofuels.

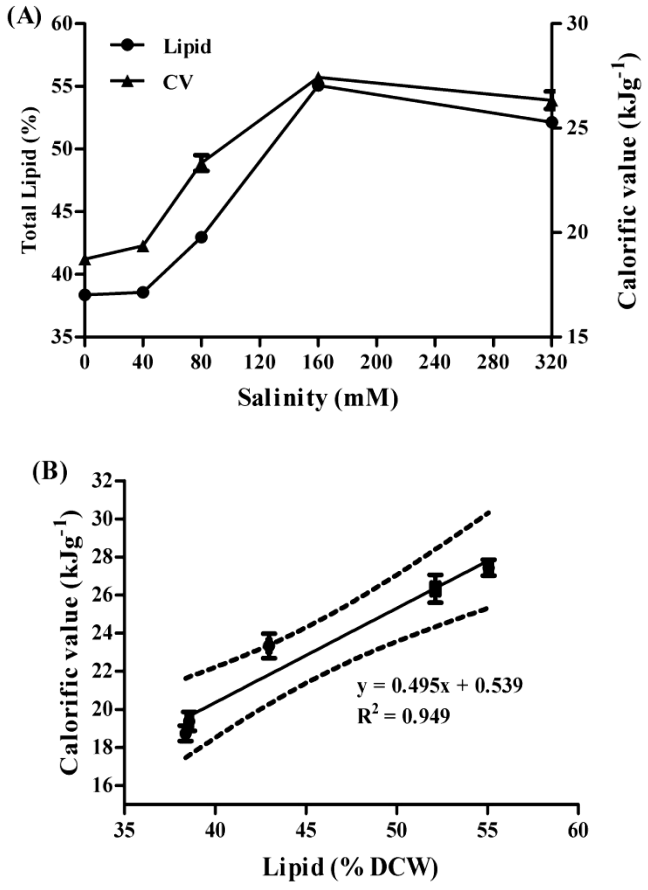

Figure - 3 (A-B)
Calorific value and total lipid content of *A.falcatus* biomass under salinity stress. (A) Increase in total lipid and calorific value with increasing salinity; (B) Linear increase in calorific value with increase in total lipid content of biomass ($R^2 = 0.949$) was observed. Error bars represent standard error (n=3)

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Reference

1. Borowitzka M. A. and Moheimani N. R., Sustainable biofuels from algae, *Mitig. Adapt. Strateg. Glob. Change*., **(2011)** (doi: 10.1007/s11027-010-9271-9)

2. Hill J., Nelson E., Tilman D., Polasky S. and Tiffany D., Environmental, economic, and energetic costs and benefits of biodiesel and bioethanol fuels, *PNAS*, **30**, 11206-11210 **(2006)**

3. Brennan L. and Owende P., Biofuels from microalgae – A review of technologies for production, processing, and extractions of biofuels and co-products, *Renew. Sust. Energ. Rev*., **14**, 557-577 **(2010)**

4. Piccolo T., Aquatic biofuels, GlobeFish-FIIU, (**2008**) (http://www.globefish.org/files/Aquaticbiofuels_638.pdf).

5. Walkar D. A., Biofuels – for better or worse?, *Ann. Appl. Biol*., **156**, 319-327 **(2010)**

6. Schenk P., Thomas-Hall S., Stephens E., Marx U., Mussgnug, J., Posten C., Kruse O. and Hankamer B., Second generation biofuels: High efficiency microalgae for biodiesel production, *BioEnerg. Res*., **1**, 20-43 **(2008)**

7. Searchinger T., Heimlich R., Houghton R. A., Dong F., Elobeid A., Fabiosa J., Tokgoz S., Hayes D. and Yu, T. H., Use of US croplands for biofuels increases greenhouse gases through emissions from land use change, *Science Express*, **1-6**, **(2008)** (doi:10.1126/science.1151861)

8. Singh A., Nigam P.S. and Murphy J.D., Mechanism and challenges in commercialization of algal biofuels, *Bioresour Technol*., **102**, 26-34 **(2010)**

9. Anandhi P. M. R., and Shaleesha A. S., Microalgae as oil producer for biofuel applications, *Res, Jour. Recent Sci*., **1(3)**, 57-62 **(2012)**

10. Chisti Y., Biodiesel from microalgae, *Biotechnol. Adv*., **25**, 294-306 **(2007)**

11. Li Q., Du W. and Liu D., Perspectives of microbial oils for biodiesel production, *Appl. Microbiol. Biotechnol.* **80**, 749-756 **(2008)**

12. Rodolfi L., Zittelli G.C., Bassi N., Padovani G., Biondi N., Bonni G. and Mario R. T., Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, *Biotechnol. Bioeng*., **102**, 100-112 **(2009)**

13. Mata T., Martins A. A. and Caetano N. S., Microalgae for biodiesel production and other applications: A review, *Renew. Sust. Energ. Rev*., **14**, 217-232 **(2010)**

14. Spolaore P., Joannis-Cassan C., Duran E. and Isambert A., Commercial applications of microalgae, *J. Biosci. Bioeng*., 101, 87-96 **(2006)**

15. Miao X. and Wu Q., High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*, *J. Biotechnol*., **110**, 85-93 **(2004)**

16. Pirt S.J., Lee Y.K., Walach M.R., Pirt M.W., Balyuzi H.H. and Bazin M.J., A tubular bioreactor for photosynthetic production of biomass from carbon dioxide: Design and performance, *J. Chem. Technol. Biotechnol.*, **33B**, 35-58 **(1983)**

17. Kosaric N. and Velikonja J., Liquid and gaseous fuels from biotechnology: Challenges and opportunities, *FEMS Microbiol. Rev*., **16**, 111-142 **(1995)**

18. Illman A.M., Scragg A.H. and Shales S.W., Increase in *Chlorella* strains calorific values when grown in low nitrogen medium, *Enzyme Microb. Technol*., **27**, 631-635 **(2000)**

19. Pienkos P.T. and Darzins A., The promise and challenges of micro-algal derived biofuels, *Biofuels Bioprod. Bioref*., **3**, 431-440 **(2009)**

20. Pokoo-Aikins G., Nadim A., EI-Halwagi M. M. and Mahalec V., Design and analysis of biodiesel production from algae grown through carbon sequestration, *Clean. Tech. Environ. Policy*, **(2009)** (doi:10.1007/s10098-009-0215-6)

21. Melis A. and Happe T., Hydrogen production, Green algae as a source of energy, *Plant Physiol*., **127**, 740-748 **(2001)**

22. Hu Q., Milton S.M., Jarvis E., Ghirardi M., Posewitz M., Seibert M. and Darzins A., Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances, *Plant J*., **54**, 621-639 **(2008)**

23. Gong Y. and Jiang M., Biodiesel production with microalgae as feedstock: From strains to biodiesel, *Biotechnol. Lett*., **33**, 1269-1284 **(2011)**

24. Clarens A.F., Resurreccion E.P., White M.A. and Colosi L.M., Environmental life cycle comparison of algae to other bioenergy feedstocks, *Environ. Sci. Technol*., **44**, 1813-1819 **(2010)**

25. Pulz O., Valuable products from biotechnology of microalgae, *Appl. Microbiol. Biotechnol*., **65**, 635-648 **(2004)**

26. Lv J.M., Cheng L.H., Xu X.H., Zhang L. and Chen H.L., Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions, *Bioresour. Technol*., **101**, 6797-6804 **(2010)**

27. Griffiths M.J. and Harrison S.T.L., Lipid productivity as a key characteristic for choosing algal species for biodiesel production, *J. Appl. Phycol*., **21(5)**, 493-507 **(2009)**

28. Abou-Shanab R.A.I., Hwang J.H., Cho Y., Min B. and Jeon B.H., Characterization of microalgal species isolated from fresh water bodies as a potential source for biodiesel production, *Appl. Energy*., **88**, 3300-3306 **(2011)**

29. Pérez M.V.J., Castillo P.S., Romera O., Moreno D.F. and Martínez C.P., Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure, *Enzyme Microb. Technol*., **34**, 392-398 **(2004)**

30. Odlare M., Nehrenheim E., Ribe V., Thorin E., Gavare M. and Grube M., Cultivation of algae with indigenous species – potentials for regional biofuel production, *Appl. Energy*., **88**, 3280-3285 **(2011)**

31. Sanchez S., Martinez M. E. and Espinola F., Biomass production and biochemical variability of the marine microalga *Isochrysis galbana* in relation to culture medium, *Biochem. Eng. J*., **6**, 13-18 **(2000)**

32. Day J. G., Slocombe S. P. and Stanley M. S., Overcoming biological constraints to enable the exploitation of microalgae for biofuels, *Bioresour. Technol*., **(2011)** (doi:10.1016/j.biortech.2011.05.033)

33. Araujo G. S., Matos L. J. B. L., Goncalves L. R. B., Fernandes F. A. N. and Farias V. R. L., Bioprospecting for oil producing microalgal strains: Evaluation of oil and biomass production for ten microalgal strains, *Bioresour. Technol*., **102**, 5248-5250 **(2011)**

34. Rao A. R., Dayananda C., Sarada R., Shamala T. R. and Ravishankar G. A., Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents, *Bioresour. Technol*., **98**, 560-564 **(2007)**

35. Reitan K. I., Rainuzzo J. R. and Olsen Y., Effect of nutrient limitation on fatty acid and lipid content of marine microalgae, *J. Phycol*., **30(6)**, 972-979 **(1994)**

36. Lebsky V. K., Gonzalez-Bashan L. E. and Bashan Y., Ultrastructure of co-immobilization of the microalga *Chlorella vulgaris* with the plant growth - promoting bacterium *Azospirillum brasilense* and with its natural associative bacterium *Phyllobacterium myrsinacearum* in alginate beads, *Can. J. Microbiol*., **47**, 1-8 **(2001)**

37. de-Bashan L. E., Bashan Y., Moreno M., Lebsky V. K. and Bustillos J. J., Increased pigment and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium *Azospirillum brasilense*, *Can. J. Microbiol*., **48**, 514-521 **(2002)**

38. Liu Y., Ruan R. and Kong Q., Mass culture of high oil content microalgae on wastewater and power plant flue gases, *Chin. J. Bioprocess Eng*., **3**, 29-33 **(2008)**

39. Knothe G., Improving biodiesel fuel properties by modifying fatty ester composition, *Energy Environ. Sci*., **2**, 759-66 **(2009)**

40. Moser B. and Vaughn S. F., Efficacy of fatty acid profile as a tool for screening feedstocks for biodiesel production, *Biomass and Bioenrgy*., **37**, 31-41**(2012)**

41. Stansell G. R., Gray V. M. and Sym S. D., Microalgal fatty acid composition: Implications for biodiesel quality, *J. Appl. Phycol*., **(2011)** (doi: 10.1007/s10811-011-9696-x)

42. Ramos M. J., Fernandez C. M., Casas A., Rodriguez L. and Perez A., Influence of fatty acid composition of raw materials on biodiesel properties, *Bioresour. Technol*., **100**, 261-268 **(2009)**

43. Boussiba S. and Vonshak A., Astaxanthin accumulation in the green algae *Haematococcus pluvialis*, *Plant Cell Physiol*., **32(7)**, 1077-1082 **(1991)**

44. Kawai H., Motomura T. and Okuda K., Algal Culturing Techniques (Ed. Anderson R. A.), Elsevier Academic Press, Burlington, MA, USA, pp. 133-134 **(2005)**

45. Levasseur M.P., Thomson A. and Harrison P.J., Physiological acclimation of marine phytoplankton to different nitrogen sources, *J. Phycol*., **29**, 587–595 **(1993)**

46. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J., Protein measurement with the folin phenol reagent, *J. Biol. Chem*., **193**, 265-275 **(1951)**

47. Hedge J. E. and Hofreiter B. T. Methods of estimating starch and carbohydrate. *In*: Carbohydrate Chemistry (Ed. Whistler R. L. and Be Miller J. N.), 17th Edition, Academic Press, New York, pp.163-201 **(1962)**

48. Bligh E. G. and Dyer W. J., A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, **37**, 911-917 **(1959)**

49. Rodríguez Ruiz J., Belarbi E. H., García Sánchez J. L. and López Alonso D., Rapid simultaneous lipid extraction and transesterification for fatty acid analyses, *Biotechnol. Techniques*, **12**, 689-691 **(1998)**

50. Abdel-Rahman M. H. M., Ali R. M. and Said H. A., Alleviation of NaCl-induced effects on *Chlorella vulgaris* and *Chlorococcum hunmicola* by riboflavin application, *Int. J. Agric. Biol*., **7(1)**, 58-62 **(2005)**

51. Hart B. T., Bailey P., Edwards R., Hortlek K., James K., McMohan A., Meredith C. and Swading K., A review of the salt sensitivity of the Australian fresh water biota, *Hydrobiologia*, **210**, 105-144 **(1991)**

52. Fodorpataki L. and Bartha C., Salt stress tolerance of a freshwater green alga under different photon flux densities,

Sludia Universities Babes-Bolyai, Biologia, **XLIX (2)**, 85-93 **(2004)**

53. Dominguez-Bocanegra A. R., Legarreta I. G., Jeronimo F. M. and Campocosio A. T., Influence of environmental and nutritional factors in the production of astaxanthin from *Haematococcus pluvialis*, *Bioresour. Technol*., **92**, 209-214 **(2004)**

54. Imamoglu E., Dalay M. C. and Sukan F.V., Influences of different stress media and high light intensities on accumulation of astaxanthin in the green alga *Haematococcus pluvialis*, *New Biotechnol*., **26**, 199-204 **(2009)**.

55. Thompson G.A. Jr., Lipids and membrane function in green algae, *Biochim. Biophys. Acta*., **1302**, 17–45 **(1996)**

56. Liu J., Huang J., Fan K.W., Jiang Y., Zhong Y., Sun Z. and Chen F., Production potential of *Chlorella zofingienesis* as a feedstock for biodiesel, *Bioresour. Technol.*, **101**, 8658-8663 **(2010)**

57. Liu J., Huang J., Sun Z., Zhong Y., Jiang Y. and Chen F., Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: Assessment of algal oils for biodiesel production, *Bioresour. Technol.*, **102**, 106-110 **(2011)**

58. El-Baky H. H. A., El-Baz F. K. and El-Baroty G. S., Production of lipids rich in omega 3 fatty acids from the halotolerant alga *Dunaliella salina*, *Biotechnology*, **3(1)**, 102-108 **(2004)**

59. Ben-Amotz A., Tornabene T. and Willium T., Chemical profile of selected species of microalgae with emphasis on lipids, *J. Phycol*., **21**, 72-81 **(1985)**

60. Tsuzuki M., Ohnuma E., Sato N., Takaku T. and Kawaguchi A., Effects of CO_2 concentration during growth on fatty acid composition in microalgae, *Plant Physiology*, **93**, 851-856 **(1990)**

61. Scragg A. H., Morrison J. and Shales S. W., The use of fuel containing *Chlorella vulgaris* in a diesel engine, *Enzyme and Microbial Technol*., **33**, 884-889 **(2003)**

62. Scragg A. H., Illman A. M., Carde A. and Shales S. W., Growth of microalgae with increased calorific values in tubular bioreactor, *Biomass and Bioenergy*, **23**, 67-73 **(2002)**

63. Bhola V., Desikan R., Santosh S. K., Subburamu K., Sanniyasi E. and Bux F., Effects of parameters affecting biomass yield and thermal behaviour of *Chlorella vulgaris*, *J. Biosci. Bioeng*., **111 (3)**, 377-382 **(2011)**