Ankistrodesmus falcatus: A promising candidate for lipid production, its biochemical analysis and strategies to enhance lipid productivity

Nitumani Kalita*, Gayatri Baruah, Rajiv Chandra Dev Goswami, Jayanta Talukdar and Mohan Chandra Kalita

Environmental Biotech Lab., Department of Biotechnology, Gauhati University, Guwahati, Assam

ABSTRACT

The present study was carried out to find out influence of different NaCl concentration (0.04 M-0.34M) in the growth of the freshwater microalga Ankistrodesmus falcatus and its biochemical constituents viz. lipid, protein, carbohydrate and secondary pigment viz. chlorophyll. There was considerable variation in growth as well as biochemical constituents of the microalga with varying concentration of NaCl. Highest increase in lipid content was found to be in 0.17 M NaCl, however protein and carbohydrate content was enhanced in 0.34M NaCl, but there was a decrease in chlorophyll content with increasing concentration of NaCl. The changes in growth and biochemical constituents indicated the influence of salinity and organism’s adaptability to the tested levels of salinity.

Key words: Microalgae, lipid, Chlorophyll, Salinity.

INTRODUCTION

Due to the shortage of fossil fuels and the production of greenhouse carbon dioxide on their combustion, alternative fuels are receiving considerable attention [1]. One of the promising alternatives is biodiesel [2]. Algae fuels present an exciting opportunity. There is a strong view among industry professionals that algae represent the most optimal feedstock for biofuel production in the long run. Continued use of petroleum-sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels [3].
While interest in algae fuels has accelerated significantly in the recent past, contrary to popular belief, research into algae as a biofuel feedstock is not new – it is over three decades old.

Algae differ in their adaptability to salinity and based on their tolerance extent they are grouped as halophilic (salt requiring for optimum growth) and halotolerant (having response mechanism that permits their existence in saline medium [4]). In either case, algae produce some metabolites to protect from salt injury and also to balance as per the surroundings osmotica [5]. *Dunaliella*, the unicellular green alga is an example for its ability to survive extreme salt stress and serve as a useful model to comprehend the strategies of cell response to high salt concentration. The present study focussed on the adaptation of the green unicellular microalgae *Ankistrodesmus falcatus* to varied range of saline condition and their effect on growth, lipid, carbohydrate, protein and chlorophyll production.

**MATERIALS AND METHODS**

2.1. Algal Culture: The strain *Ankistrodesmus falcatus* was obtained from the freshwater microalgae culture laboratory of the Department of Biotechnology, Gauhati University. The algal strain was maintained in its purified form by subsequent sub culture in BG11 media in every 3 weeks.

2.2. Media and Culture condition: For comparative growth study two autotrophic media were used - Blue Green Algal medium 11 (BG11) (Adapted from [6] taken from SAG culture media receipt) and Bold’s Basal Medium (BBM) [7]. For analysis of NaCl effect on growth, 120 ml of BBM media was distributed in a set of twelve 500 ml Erlenmyer conical flask and sodium chloride was added in the range of 0.04M, 0.08M, 0.17M & 0.34 M to the media in the flasks and inoculated with 30 ml of 3 weeks old culture of *Ankistrodesmus falcatus* (20% v/v). The culture flasks were incubated at 25°C and maximum 29±1°C under 1.5- 2.0 Klux light intensity and 16:8 h light dark cycle. All the experiments were carried out in triplicates and average values were recorded at specific time intervals.

2.3. Analytical methods:

2.3.1 Growth study - Growth of the selected microalgae species was analyzed from the semi continuous culture over a period of 20-24 days of culture time. For growth analysis sampling was done (10 ml from each replicates) at specific intervals of culture age and growth of the microalga was estimated by cell count and optical density of the culture at 725nm.

2.3.2 Biochemical analysis: To determine the total lipid, carbohydrate and protein content quantitatively, definite volume of cultures of the microalgae species were harvested at mid log phase by centrifugation. The cells were lyophilized and stored for further biochemical analysis.

2.3.2.1 Quantitative Estimation of Total Lipid, (The extraction mechanism and analysis): Lipid estimation was carried out following [8] with some modifications. Known amount (100mg) of dried biomass was homogenized with chloroform: methanol 1:2 (v/v) at 37°C and the extract was then centrifuged at 10000 rpm for 5 minutes. The supernatant was collected in a separating funnel. The residue was further homogenized with chloroform and centrifuged to collect the supernatant. The filtrate was washed with 0.9% sodium chloride (NaCl) solution; the
lower layer of chloroform was then separated and treated with anhydrous sodium sulphate (Na₂SO₄) to remove the traces of water. The lipid content was determined gravimetrically and expressed as dry weight percentage after evaporating chloroform.

2.3.2.2 Quantitative Estimation of Protein and carbohydrate: Protein estimation was carried out following Lowry’s Method [9]. It is a sensitive method, which determines the protein content of enzyme extracts. A blue colour develops in the reaction by the reduction of phosphomolybdic-phosphotungstic component of Folin–ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein.

The carbohydrate was estimated according to Anthrone method [10]. Carbohydrate was first hydrolyzed into simple sugars with hydrochloric acid. In hot acidic medium the glucose components were dehydrated to hydroxymethyl furfural, which gives green coloured product with anthrone. The experiment was done in triplicates.

2.3.2.3 Analysis of chlorophyll pigment: To estimate the amount of chlorophyll, known volume of Ankistrodesmus falcatus culture (10 ml for each replicates) was centrifuged (8000rpm) for 10 min. Then the supernatant was discarded and the pellet was treated with known volume of acetone and kept in the refrigerator at 4°C overnight. The chlorophyll content in the pooled extract was estimated spectrophotometrically by recording absorbance at 663nm and 645nm for chlorophyll a and chlorophyll b and quantified using the method of [11].

**Calculation:**

The concentration of chlorophyll a (mg l⁻¹):

\[ C_a = 12.25 \times D_{663\text{ nm}} - 2.79 \times D_{647\text{ nm}} \]

The concentration of chlorophyll b (mg l⁻¹):

\[ C_b = 21.5 \times D_{647\text{ nm}} - 5.1 \times D_{663\text{ nm}} \]

**RESULTS**

3.1 Comparative growth rate analysis

For comparative analysis the growth behaviour of the strain was assessed in two different media viz. Blue Green Algae Medium (BG 11)- media which does not contain NaCl as a constituent and Bold’s Basal Media (BBM)- media that contain 0.04 M NaCl as a constituent, under laboratory conditions as stated under materials and method. For this experiment 20% of exponentially growing culture containing cell numbers of 4.5 x 10⁵ cells ml⁻¹ was inoculated into each of the three replicates of the two media. The optical density of the species in two different media was determined at 725 nm absorbance using U. V. spectrophotometer.

It was observed that the species showed a better growth in the BBM media in comparison to BG11 media showing the best result in terms of number of cells per ml of the media and cell density. Results reveal that the steady phase of growth starts from the 21st day of culture in case of BG11 media and 24th day of culture in case of BBM media. It was also observed that the log
phase started from the second week of culture in case of both BBM and BG11 media (Fig.1.a and 1.b). It can be seen in the Figure: 1 that the growth curve becomes horizontal from the 21st day from the date of starting experiment in case of BG11 and 24th day from the date of starting experiment in case of BBM. Though the culture was started with the same inoculums, the cell growth varied in the two media. This variation might be due to the different nutrient composition of different media.

**Fig1:** Growth curve of *Ankistrodesmus falcatus* in BG11 and BBM medium: (a) Cell count and (b) spectroscopic method (725nm)
3.2.1 Effect of NaCl on the growth rate of *Ankistrodesmus falcatus*

### 3.2.1.1 Comparative growth study of *Ankistrodesmus falcatus* by cell count method

The comparative growth characteristics of the species *Ankistrodesmus falcatus* was determined by counting cell numbers over a period of 20 days in BBM media with four different concentration of NaCl - 0.04M, 0.08 M, 0.17 M, 0.34 M and the comparative growth curves for cell number of *Ankistrodesmus falcatus* in the four media are given in the Fig.2.a.

The growth curves showed that the species was with the best growth in BBM media that contained 0.17 M NaCl.

---

**Fig.2.a:** Comparative Growth curves of *Ankistrodesmus falcatus* in BBM media in four different concentration of NaCl by cell count method.

**Fig.2.b:** Comparative Growth curves of *Ankistrodesmus falcatus* in BBM media in four different concentration of NaCl by spectroscopic method (725nm)
3.2.1.2 Comparative growth study of Ankistrodesmus fulcatus at different concentration of NaCl at 725 nm absorbance:
The comparative study of the optical densities of *Ankistrodesmus fulcatus* in media containing different NaCl concentration also showed the same result, with 0.17M NaCl modified media showing better result compared to other media (Fig.2.b).

3.2 Biochemical Analysis:
3.2.1 Determination of Biochemical Content of the species in BBM media
The total lipid, protein, carbohydrate components of *Ankistrodesmus fulcatus* were estimated by following standard methodologies as described in the materials and methods. The cultured cells were harvested at mid log phase through centrifugation and pellets were lyophilized and the total biochemical content in % dry cell weight was determined gravimetrically. It has been found that the % dry cell weight of lipid content is highest in the strain while lowest is the protein (Fig. 3).

![Biochemical constituents](image)

**Fig 3:** Comparison of percentage (%) dry cell weight values of total lipid, total protein and total carbohydrate

![Media](image)

**Fig 4.a:** Percentage (%) dry cell weight values of total lipid content in different NaCl concentration,
3.2.2 Effect of NaCl on Biochemical constituents of the microalgae *Ankistrodesmus falcatus*

The biochemical compositions were also examined in the four different media from the dried biomass of the cultures harvested at mid exponential phase. The comparison of biochemical contents is represented in the Fig. 4.a, 4.b and 4.c.

### 3.2.2.1 Total lipid content

The highest lipid content was estimated in the BBM media with 0.17M NaCl, followed by 0.34M NaCl and BBM media with 0.08M NaCl. The least amount of total lipid was estimated in BBM media with 0.04M NaCl. (Fig 4.a)

### 3.2.2.2 Total carbohydrate content

Among the four different NaCl concentration used, the highest carbohydrate content was estimated in 0.34M NaCl, followed by 0.17M NaCl and 0.08M NaCl. The least amount of carbohydrate was found to be in 0.04M NaCl (Fig 4.b).

### 3.2.2.3 Total protein content

Among all the media studied, the highest protein content of the species was estimated in BBM media with 0.34M NaCl and 0.04M NaCl showed least protein content (Fig 4.c).
3.2.2.4 Effect of NaCl on Chlorophyll content of *Ankistrodesmus falcatus*

The chlorophyll content (chlorophyll- a and chlorophyll- b) of *A. falcatus* was estimated at mid log phase by harvesting cells of 5 ml of culture. Highest chlorophyll content was recorded in BBM media with 0.04M NaCl and least amount of chlorophyll was recorded in 0.34M NaCl (Fig- 5).

![Fig.5. Chlorophyll a and b content of *Ankistrodesmus falcatus* (mg/ml) in different NaCl concentration](image)

**DISCUSSION**

In the present study, it was seen that the freshwater Chlorophycean strain *Ankistrodesmus falcatus* showed better growth in Bolds Basal Media in comparison to BG11 media ($9.7 \times 10^5$ cells/ml and $9.0 \times 10^5$ cells/ml respectively) on 21st days of culture. Similar result was found in case of the green alga *Haematococcus pluvialis* that showed maximum cell growth on BBM media but the growth was much lower in BG11 media ([12], [13]). But in case of the fresh water green alga *Botryococcus braunii* BG11 was found to be the best media [14].

*Ankistrodesmus falcatus* in the present study was able to grow in all the tested concentrations of sodium chloride (0.04M to 0.34M). *A falcatus* showed enhanced growth rate up to an optimum NaCl concentration with highest growth at 0.17M NaCl but when the concentration was increased beyond that level (0.34M), the growth was found to decrease. *B. braunii* when tested in different NaCl concentration (17mM to 85mM) showed highest growth in 17mM and 34mM concentration [4]. However, [15] showed the reduced growth of *Botryococcus braunii* as NaCl concentration was increased due to decrease in photosynthetic rate. The growth of *Chlorella vulgaris* and *Chlorococcum hunicola* was found to significantly increase at low level of salinity (50mM to 100mM) but with rise in NaCl (200mM and 250mM) concentration the growth remained steady in case of *Chlorella vulgaris* but decreased in *C. humicola* [16]. In the present study of *A. falcatus* also the growth was found to increase at low salinity level (0.08M to 0.17M) and decreased with rise in NaCl concentration (0.34M). The salinity-induced growth reduction may be attributed to the accumulation of reactive oxygen species [17].

Environmental factors are known to influence lipid production by microalgae. One of the factors influencing lipid content of algae and causing its increase is salinity. In the present study highest lipid content was recorded in 0.17M NaCl and least amount was recorded in 0.04M. There was found to be an increase level of lipid content with increasing salinity. The result showed that
there is about 8-13% increase in lipid content with increasing NaCl concentration in the range of 0.25-1%. Similar result was reported by [18] showing that increasing NaCl concentration ranging from 0-3% resulted in an increase lipid content from 34-46%. Cells of B. braunii cultured in the presence of low NaCl concentration (0.17-0.85mM) also contained enhanced amount of lipid [4]. However, [19] reported that lipid content remained same in cells of two B. braunii strains cultured in the presence and absence of NaCl. In the halotolerant green alga Dunallella tertiolecta, salt stress causes an increase in the intracellular lipid content ([20] and [21]). Thus NaCl has been found to influence alteration in lipid content of algal cells. In case of A. falcatus also lipid content is fond to vary with different NaCl concentration. Many reports have suggested that lipids might be involved in the protection against salt stress ([22], [23], nd [24]). Alteration in the lipid content of membranes of an organism is of major importance in response to environmental stresses ([25], [24]). Carbohydrate content in A. falcatus was found to increase with increasing concentration of NaCl. Maximum amount was found in 0.34M and least amount in 0.04M NaCl. In B. braunii also increasing NaCl concentration resulted in increase in amount of carbohydrate [4]. The alga B. braunii produced carbohydrate as an osmoprotectant as reported by [19] with increasing salinity was also in accordance with the present results. Protein content of the microalgae was found to increase with increasing NaCl concentration. Maximum was recorded in 0.34M while minimum was recorded in 0.04M concentration of NaCl. This result is probably because of the synthesis of new proteins in response to increased salinity level [26].

There was slight decrease in chlorophyll content with increasing salt concentration. Least amount of chlorophyll was recorded in 0.34m NaCl. In case of Tetraselmis chuii also chlorophyll content was found to decrease with increasing NaCl concentration [27]. Due to increasing salinity level the cell usually get depleted of water, which affect photosynthesis. Thus chlorophyll content may get decreased with increasing NaCl concentration.

Thus from our research findings, the microalgae Ankistrodesmus falcatus is found to be quite promising in terms of sustainable energy production, as a high lipid content was reported from various research findings, proper utilization and mass production strategies of this species may lead to development a better way for production of alternative fuel, which opens up new vistas for biofuel technologies.

Acknowledgements
We are grateful to the Head of the Department, Department of Biotechnology, Gauhati University for providing facilities for the work. We gratefully acknowledge Dr. Hridip Kumar Sarma of the Department of Biotechnology for his help during the experiments. Finally we also acknowledge Rituparna borah and Pranjan Barman for his help during the entire course of the work.

REFERENCES

Available online at www.scholarsresearchlibrary.com