Biomass, lipid and fatty acid composition of two filamentous green algae, *Spirogyra punctulata* and *Ulva intestinalis* under various concentrations of NaCl

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Abstract

The effects of various concentrations of NaCl on biomass yield, intracellular lipid accumulation and fatty acid profile of the high lipid containing cells of the brackish water filamentous green algae Spirogyra punctulata and Ulva intestinalis in different culture phases were investigated. The highest biomass obtained for S. punctulata and U. intestinalis was 2.19±0.28 g/L (on 10th day) and 3.78±0.03 g/L (on 12th day) under 0.05 g/L and 0.5 g/L NaCl respectively ($p<0.05$). The two-fold increase of total lipid accumulation in S. punctulata (32.56±0.35%) and U. intestinalis (36.15±0.34%) was studied under 0.05 g/L NaCl on 8th day of cultivation. High lipid containing cells at 0.05 g/L NaCl showed enhancement of saturated and monounsaturated fatty acids than polyunsaturated fatty acids when compared to control. Among the individual fatty acids, palmitoleic, stearic and oleic acids were found significantly higher than the control. Palmitic acid in S. punctulata decreased but increased in U. intestinalis with higher concentrations of NaCl ($p<0.05$).

However, α-linoleic, paullinic, arachidonic, eicosapentaenoic and lignoceric acids were newly observed in NaCl induced cultures of U. intestinalis which were completely absent in control cultures. Therefore, the present study suggests that high biomass, lipid and fatty acid synthesis of S. punctulata and U. intestinalis could be the possible outcome for bioenergy research.

Keywords: Biomass, Fatty acid, Filamentous algae, Lipid, NaCl, Stress.

Introduction

Algae have the capacity to compete with conventional crops for better biomass productivity which ultimately leads to the production of lipids as bioenergy resource. However, most of the recent developments are techno-economic for the production of large-scale biomass in downstream process; still these are not economically feasible because of the high production cost[10,16]. To replace the conventional fossil fuel, large quantities of lipid rich oleaginous micro- and macroalgal biomass are needed which can make algae based cost-effective biofuels. Therefore, researchers have tried to find a way for cost-effective biofuel production through the modification of culture conditions in photobioreactors and open raceway ponds[7,27].

In particular, the environmental factors especially the abiotic factors can stimulate hyper-accumulation of lipid in algae[13,20,21]. Till date, a number of stress factors like nutrients, heavy metals, light intensity, temperature, pH etc. have been explored to induce biomass and high lipid accumulation in algal cells[2,19]. Salinity is one of the important stress factors that induces growth and hyper-accumulation of lipid in algal cells at elevated concentrations. Most of the researchers have done their work on growth and lipid induction in microalgae such as *Botryococcus braunii*, *Scenedesmus obliquus*, *Dunaliella salina*, *Chlorella vulgaris*, *C. sorokiniana*, *C. ellipsoidea*, *Chlorococcum infusionum* etc. using NaCl as stress factor[1,5,9,10,18,26].

Low cost recovery in large-scale cultivation and high resistance to grazer predation are the two important criteria for choosing filamentous algae compared to unicellular microalgae. Some oleaginous filamentous macroalgae such as *Oedogonium nodulosum*, *Zygnema extenue*, *Stigeoclonium* sp. and *Hormidium* sp. have shown positive output in growth and lipid accumulation when cultivated in large scale[25]. Among the nutrients, nitrogen starvation resulted high lipid productivity (45.38% in dry weight) in *Hormidium* sp. and *Oedogonium nodulosum* which could be substitute for biodiesel and bioethanol production[25]. Very few reports are available on filamentous micro- and macroalgae for growth and lipid accumulation study under salt stress.

Lawton et al[12] have worked on *Oedogonium* sp. to observe the effect of salinity on biomass productivity, protein and lipid composition. Moreover, marine macroalgae can tolerate a wide range of salinities for growth and other biochemical activities. Salinity at the range of 20-40 ppt (parts per thousand) can stimulate hydrocolloid production in *Gracilaria chilensis*[12].

Furthermore, *Porphyra umbilicalis* grows well in high salinity ranging from 7-52 ppt[11]. In another analogous study, de Paula Silva et al[6] have reported that *Chaetomorpha indica* and *Ulva ohnoi* can grow better in salinities of 5-45 ppt. It has been reported that some freshwater filamentous algae

such as *Cladophora*, *Pithophora*, *Microspora*, *Oedogonium* and *Micractinium* can grow in aquatic environment with limited nutrients[22].

Therefore, the objective of this study was to assess the effect of various salt (NaCl) concentrations on biomass and lipid accumulation in *S. punctulata* and *U. intestinalis* and to determine the fatty acids changes in high lipid containing cells grown under optimum NaCl.

Material and Methods

Algal strain: The green filamentous alga *S. punctulata* was collected from Dobanki camp (N 21º42.346', E 088º18.899') with salinity 9.2 ppt while *U. intestinalis* was collected from Fraserganj (N 20º03.031', E 088º81.310') with salinity 12.7 ppt. Both the algal species were collected from the brackish water habitats of Indian Sundarbans. Collected species were properly identified and submitted in Calcutta University Herbarium (CUH).

Culturing: The algal cells were initially maintained and cultivated in Bold Basal Medium (BBM) at 20±2ºC with light illumination of 2000 lux.[4] The algae were cultured in 500 ml flask with 16:8 light-dark cycles. The cultures were continuously aerated with 1% (v/v) CO_2 at 135rpm agitation in Eyela horizontal shaker-incubator (Eyela, Japan)[17]. The modified BBM was used with tested NaCl concentrations of 0, 0.025 (control), 0.05, 0.1, 0.5, 1, 1.5, 2 and 2.5 g/L.

Gravimetric determination of Biomass and Lipid: Biomass yield (g/L) in terms of dry cell weight (dcw) was measured gravimetrically. For growth measurement, algal cells were harvested on day 0 and subsequently after every 2 days interval for 30 days. For harvesting, algal biomass were filtered through filter paper and dried in open air for 2-3 days. Sun drying is the best method for filamentous algae over other drying methods as it is convenient, efficient and cost-effective[19,20].

For extraction and quantification of lipid under different concentrations of NaCl, the pre-harvested algal biomass was taken and dried further at 70ºC for 2 hours in oven. The completely dried biomass was grinded with mortar and pestle to make the powder. The lipid was extracted from powdered biomass using chloroform and methanol in the ratio of 2:1 (v/v) following the protocol of Bligh and Dyer[3]. The percentage of lipid in dry cell weight was determined from the pre-weighed vials used in rotary evaporator at constant temperature.

Transesterification and FAME analysis: The FAME was prepared using high lipid containing cells grown under 0.05 g/L NaCl and compared with the control (0.025 g/L NaCl). The acid catalyzed transesterification reaction at 60ºC using lipid, methanol and hydrochloric acid (HCl) in the molar ratio of 1:80:4 and incubated for 6.4 hours. The upper oil containing organic phase was pipetted out and taken for gas chromatography mass spectrometry (GC-MS) analysis using Agilent 6890N Gas Chromatograph connected to an Agilent 5973 Mass Selective Detector at 70 eV. The HP-5 MS capillary column (30m × 0.25mm i.d. × 0.25µm film thickness) was equipped with the instrument. The identification of fatty acid class was determined on the basis of retention time[19,20].

Statistical analysis: Statistical analysis was carried out in triplicate (n=3) and data presented as mean±standard deviation (SD) with standard error (SE) bars. Significant differences (p<0.05) between means in terms of biomass, lipid and fatty acids were obtained through one-way analysis of variance (ANOVA) and Tukey's test.

Results and Discussion

Effect of NaCl on biomass yield: Biomasses obtained from three replicates of each culture were analyzed at 2 days interval for 30 days. Although, *S. punctulata* and *U. intestinalis* are brackish water strains, it was interesting to study their tolerance level to various concentrations of NaCl in order to achieve better growth. As demonstrated in fig. 1, growth of the cells in terms of biomass of *S. punctulata* under 0, 0.025, 0.05, 0.1, 0.5, 1, 1.5, 2 and 2.5 g/L NaCl for 30 days showed the maximum biomass value of 2.19±0.28 g/L at 0.05 g/L NaCl on 10th day of cultivation (p<0.05). In *U. intestinalis*, maximum biomass was achieved (3.78±0.03 g/L) at 0.5 g/L NaCl on 12th day of cultivation (Fig. 2). Biomass yield for *S. punctulata* and *U. intestinalis* was 1.6 and 1.4 times better than control.

The maximum tolerance level of NaCl was determined as 0.05 and 0.5 g/L for *Spirogyra* and *Ulva* respectively. The negative trend in growth was observed when the NaCl concentrations were further increased to 0.1 g/L for *Spirogyra* and 1 g/L for *Ulva.* From another results, it can be suggested that low to medium salinity between 0 to 0.05 M NaCl, was appropriate for better biomass in *S. obliquus*[9].

The osmotic potential of the surrounding culture medium was counter balanced with varied concentrations of NaCl. It has been reported that sodium ion (Na^+) in osmolyte may sequester in vacuole to maintain the osmotic pressure (turgor pressure) in algal cells during high salinity[9]. The decrease in growth and biomass yield with increasing salinity has been reported in many studies on macro- and microalgae[12,18]. The rapid decrease of growth in high salinity is due to available energy through osmoregulation but not due to photosynthesis and cell division[12].

Furthermore, the reactive oxygen species (ROS) were generated in high salt stress conditions, which lead to slow growth[14]. The growth of algal cells can be achieved with low salt concentrations while the degradation of chlorophyll can lead to cell death at high salt concentrations. Meanwhile, an important study has suggested that with changing NaCl concentrations, the salt tolerant enzymes can promote the growth of the microalga under various salinity level[14].

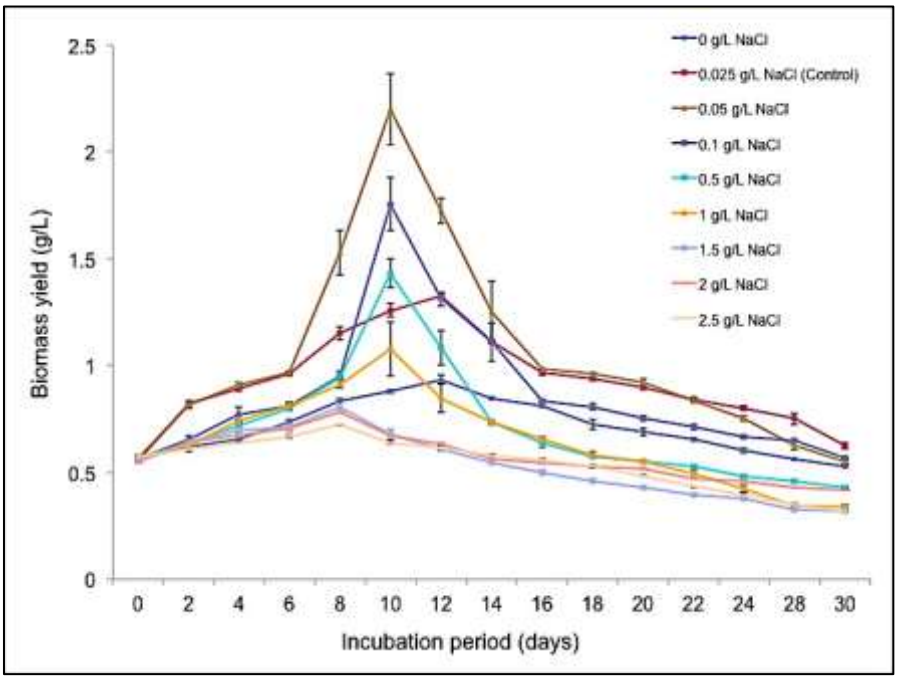

Fig. 1: Biomass yield (g/L) of *S. punctulata* under various concentrations of NaCl (Results demonstrated as mean ± SD, $p<0.05$)

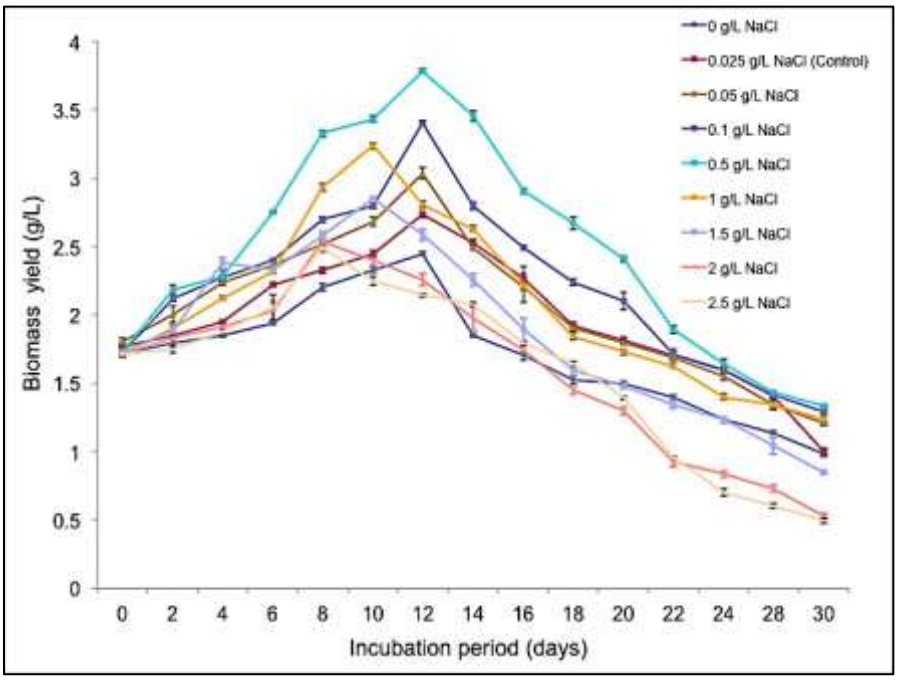

Fig. 2: Fig. 1- Biomass yield (g/L) of *U. intestinalis* under various concentrations of NaCl (Results demonstrated as mean ± SD, $p<0.05$)

The growth rate and photosynthesis in filamentous micro- and macroalgae are typically found to be higher in high salinity because they are predominant in natural environment like ponds, lakes, fisheries and seashores.

Effect of NaCl on lipid accumulation: In order to know the effect of NaCl on lipid production and accumulation, *S. punctulata* and *U. intestinalis* were subjected to a range of salinity gradient. Lipid accumulation of *S. punctulata* and *U. intestinalis* grown in medium containing various concentrations of NaCl was almost found better than those of the control cultures on or after 8 days of the experiment. Furthermore, the lipid accumulation in *S. punctulata* and *U. intestinalis* was 32.56±0.35% and 36.15±0.34% at 0.05 g/L on 8th day of experiment, which was 2 times better than control.

With the increasing concentrations of NaCl greater than 0.05 g/L after 8 days of cultivation, a sharp decline in lipid accumulation was observed (Figs. 3-4). In preliminary

experiments, we found that *S. punctulata* and *U. intestinalis* were not able to accumulate high percentage of lipid (>30%) at 0 and 0.025 g/L NaCl. Significant differences (p<0.05) were found in lipid accumulation of *S. punctulata* and *U. intestinalis* between the control and the cultures containing different concentrations of NaCl. Reports on lipid accumulation under salt stress in filamentous micro- and macroalgae are available in few studies[12,24].

A contradictory report by Elenkov et al[8] showed that elevated level of salinity could decrease the lipid accumulation in *Cladophora vagabunda*. In contrast, *Oedogonium* sp. and *Tribonema minus* resulted in high lipid accumulation under elevated concentrations of NaCl[12,24]. Nutrient limitation is one of the most promising abiotic stress factors used by many researchers to change the biochemical pathways and cell cycle of algae linked to lipid accumulation[13,15,27]. It has been suggested that the effect of high Na^+ concentrations in the culture in presence of depleted nitrogen stimulates lipid synthesis and accumulation[5].

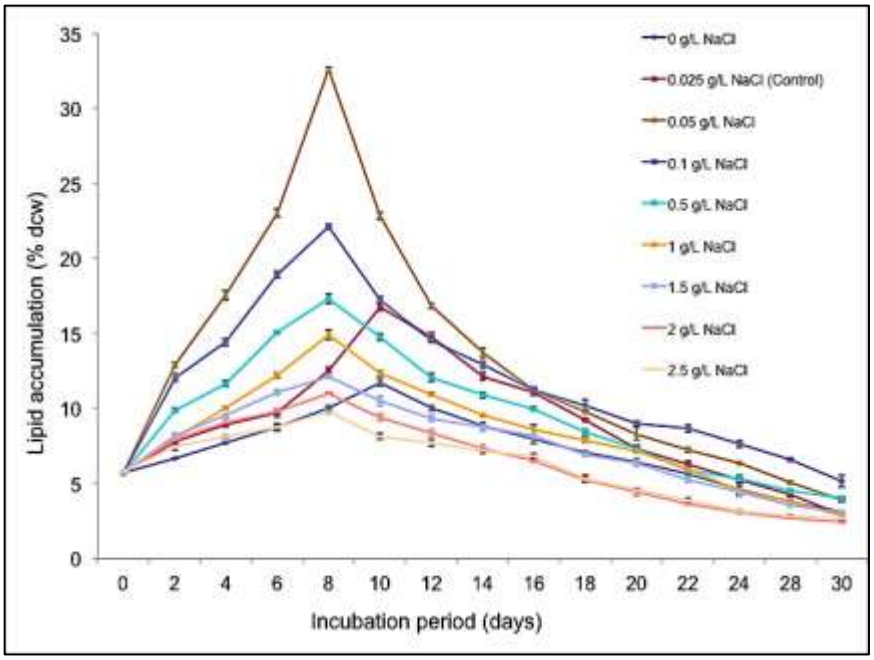

Fig. 3: Lipid accumulation (% dcw) in *S. punctulata* under various concentrations of NaCl (Results demonstrated as mean ± SD, p<0.05)

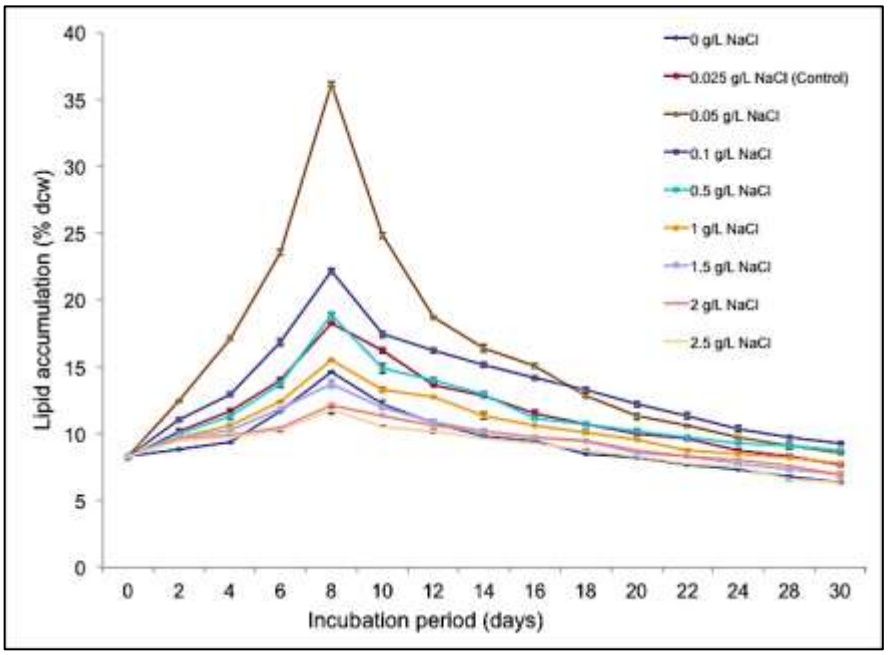

Fig. 4: Lipid accumulation (% dcw) in *U. intestinalis* under various concentrations of NaCl (Results demonstrated as mean ± SD, p<0.05)

In microalga, *S. obliquus*, oil accumulation was enhanced by 36% when supplemented with 0.3 M NaCl after 15 days of experiment. This is in agreement with the results of our study using *U. intestinalis*. On the other hand, *Chlorella* sp. showed maximum lipid accumulation of 21.4% at 0.5 M NaCl which is 1.6 times more than the control cultures[14].

In our previous study, we have reported that increased concentrations of NaCl did not affect the growth and lipid accumulation in *C. ellipsoidea* but slowed the process in *C. infusionum*[18]. Total lipid accumulation in *C. ellipsoidea* was 45.8±0.8% at 5 g/L NaCl, while in *C. infusionum*, it was 36.33±0.56% at 1.5 g/L NaCl[18]. Under salt stress, the oxidized form of nicotinamide adenine dinucleotide phosphate ($NADP^+$) becomes depleted that alters the lipid biosynthetic pathway in algae and higher plants.

Furthermore, two major enzymes, acetyl CoA carboxylase (ACCase) and fatty acid synthase (FAS) can regulate the lipid and fatty acid biosynthesis. It has also been suggested that in lipid biosynthesis, the activity of ACCase may increase under NaCl which promotes the accumulation of adenosine tri-phosphate (ATP) and $NADP^+$ leading to synthesis acetyl-CoA to malonyl-CoA[18].

Effect of NaCl on FAME composition: High lipid containing biomass on 8th day of cultivation at 0.05 g/L NaCl was taken for FAME analysis and compared with the control (Table 1). The major fatty acids obtained in the studied algae were grouped into SFA, MUFA and PUFA (Fig. 5). The significant results (p<0.05) in fatty acid compositions were obtained for both the studied algal species containing a total number of 17 fatty acids. The high percentage of C16 and C18 fatty acids was obtained from control and salt stressed culture of *S. punctulata* and *U. intestinalis*. The similar results were obtained in *B. braunii* IPPAS H-252[26]. High proportions of palmitic acids (C16:0) in control and experimental sets were consistent with our previous data[18].

Subsequently the enhancement of palmitoleic acid to 8.55±0.35% in *S. punctulata* and 12.25±0.35% in *U. intestinalis* was observed under 0.05 g/L NaCl which was 3.2 and 1.2- fold better than control. Furthermore, significant (p<0.05) results were also obtained for stearic and oleic acids in the experimental cultures. In *S. punctulata*, reduction of linoleic and α-linoleic acids was observed under NaCl stress and decreased upto 1.46 and 3.35 times than control. Interestingly, the linoleic acid in *U. intestinalis* was not detected under high NaCl, while it was present in control cultures. Moreover, the α-linoleic acid in *U. intestinalis* reappeared in high NaCl but was absent in control biomass.

At the same time, margaric acid in *U. intestinalis* and dihomo-γ-linolenic acid in *S. punctulata* were not detected throughout the experiment. With the increasing concentrations of NaCl in culture, the trienoic acids C16:3 and C18:3 decreased significantly[26].

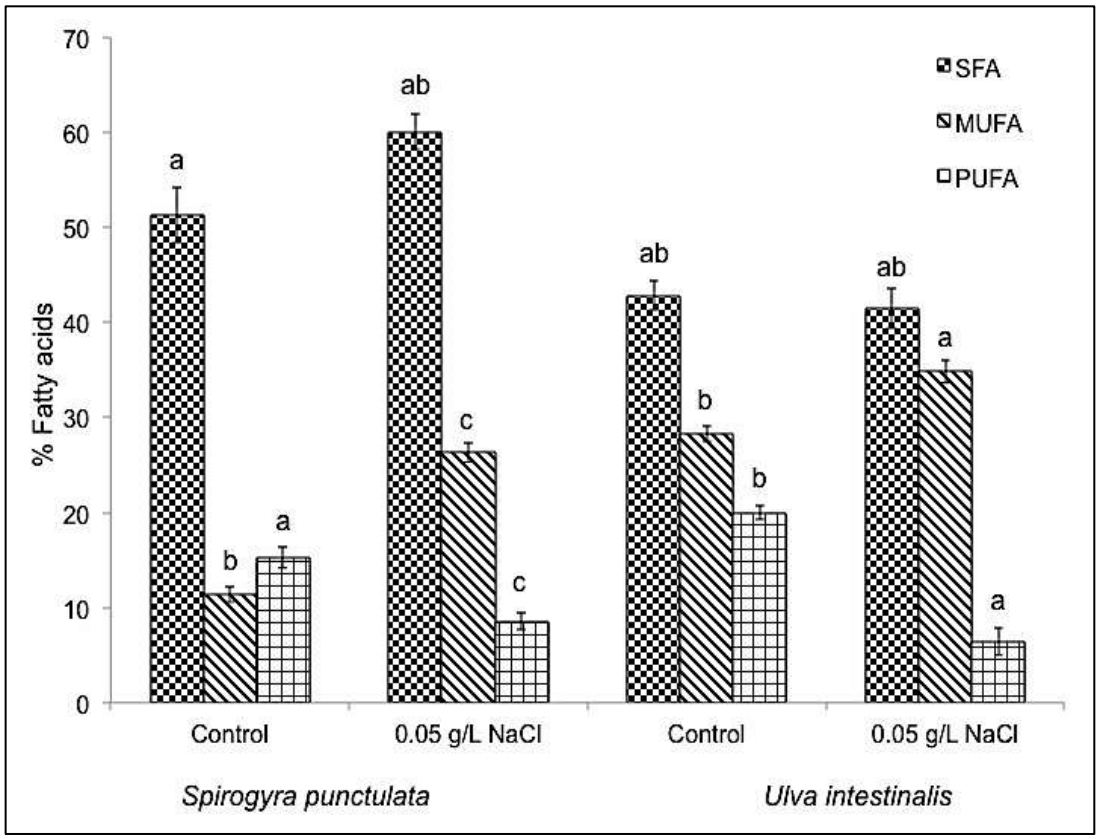

Fig. 5: Percentage (%) fatty acids of SFA, MUFA and PUFA under control and optimum NaCl concentrations (Results demonstrated as mean ± SD, Lowercase letters indicate significant differences (p<0.05) for each treatment)

Table 1
Fatty acid compositions of *S. punctulata* and *U. intestinalis* under control and optimum concentrations of NaCl.

Fatty acids	Fatty acid compositions (%)			
	S. punctulata		*U. intestinalis*	
	Control	0.05 g/L NaCl	Control	0.05 g/L NaCl
Lauric (C12:0)	0.85±0.35^{b}	0.65±0.21	4.51±0.28^{bc}	1.75±0.21^{c}
Myristic (C14:0)	1.95±0.35^{b}	1.01±0.42^{ab}	9.72±0.28^{ab}	1.51±0.28
Pentadecylic (C15:0)	1.05±0.35	0.75±0.21	ND	0.85±0.07^{a}
Palmitic (C16:0)	37.9±0.28^{ab}	36.75±0.21^{a}	22.60±0.14^{a}	25.50±0.21^{ab}
Palmitoleic (C16:1)	2.65±0.21	8.55±0.35^{a}	9.50±0.28^{ab}	12.25±0.35^{b}
Margaric (C17:0)	4.15±0.35^{a}	6.10±0.28^{bc}	ND	ND
Stearic (C18:0)	2.01±0.42	7.95±0.35^{c}	4.61±0.42	8.85±0.49^{c}
Oleic (C18:1)	8.10±0.42^{a}	16.05±0.35^{ab}	18.80±0.56^{a}	20.25±0.49^{bc}
Linoleic (C18:2)	5.50±0.28^{b}	3.75±0.21	15.05±0.35^{ab}	ND
α-linoleic (C18:3)	8.90±0.42^{b}	2.65±0.21	ND	1.01±0.42
Arachidic (C20:0)	0.61±0.28^{bc}	ND	0.75±0.21	0.65±0.21
Paullinic (C20:1)	0.65±0.21	1.70±0.28	ND	2.35±0.35^{ab}
Dihomo-γ-linolenic (C20:3)	ND	ND	4.95±0.35^{c}	2.60±0.42
Arachidonic (C20:4)	0.25±0.21	1.45±0.21^{bc}	ND	1.31±0.28^{c}
Eicosapentaenoic (C20:5)	0.65±0.21	0.70±0.28	ND	1.55±0.35^{bc}
Behenic (C22:0)	1.60±0.14	0.65±0.07^{b}	0.65±0.21	0.75±0.21^{a}
Lignoceric (C24:0)	1.20±0.42	6.20±0.14^{c}	ND	1.60±0.42

Values expressed as mean ± SD (n=3). Superscript letters indicate significant differences (p<0.05) for each treatment. ND: Not detected.

The 5-fold increase of lignoceric acid was observed in *S. punctulata* under NaCl stress. The proportions of pentadecylic acid (C15:0), paullinic acid (C20:1), arachidonic acid (C20:4) and eicosapentaenoic acids (C20:5) were newly formed in NaCl treated culture of *U. intestinalis* which were completely absent in control.

Moreover, the amount of SFA was more than 50% of the total fatty acids in *S. punctulata* while it was just 50% or less in *U. intestinalis* (Fig. 5). Simultaneously, the highest MUFA content was achieved in NaCl treated cultures of *S. punctulata* and *U. intestinalis* which was 2.3 times and 1.2 times better than control. For both the algae, the PUFA content decreased significantly with increasing NaCl in the culture which can be suggested for biodiesel application. Intermediate acyl carrier protein (ACP) and FAS can stimulate free fatty acid (FFA) formation in cytoplasm which leads to form membrane polar lipids and storage triacylglycerol (TAG) in algae and higher plants[18].

Conclusion

Rising sea water level and continuous mixing of saline water into fresh and brackish water habitats can create a significant global problem in agriculture. It can be assumed that more than 50% of the arable lands will be affected by saline water by the year 2050.[27] To overcome this situation, salt tolerant filamentous micro- and macroalgae would be the best

possible outcome for sustainable biomass without impacting the agriculture.

Our study has demonstrated that *S. punctulata* and *U. intestinalis* can grow vigorously under various concentrations of NaCl. High biomass yield and lipid accumulation have suggested that *S. punctulata* and *U. intestinalis* can be the best candidates for bioenergy research. Alternatively, good quality fatty acids produced by these species can be subjected to food, fodder and biodiesel.

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References

1. Ahmed R.A., He M., Aftab R.A., Zheng S., Nagi M., Bakri R. and Wang C., Bioenergy application of *Dunaliella salina* SA134 grown at various salinity levels for lipid production, *Sci. Rep.*, **7**, 8118 **(2017)**

2. Aratboni H.A., Rafiei N., Garcia-Granados R., Alemzadeh A. and Morones-Ramirez J.R., Biomass and lipid induction strategies in microalgae for biofuel production and other applications, *Microb. Cell Fact.*, **18**, 178 **(2019)**

3. Bligh E.G. and Dyer W.J., A rapid method of total lipid extraction and purification, *Canadian J. Biochem. Physiol.*, **37(8)**, 911-917 **(1959)**

4. Bold H.C., The morphology of *Chlamydomonas chlamydogama* sp. nov., *Bull. Torrey Bot. Club*, **76(2)**, 101-108 **(1949)**

5. Church J., Hwang J.H., Kim K.T., McLean R., Oh Y.K., Nam B., Joo J.C. and Lee W.H., Effect of salt type and concentration on the growth and lipid content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production, *Bioresour. Technol.*, **243**, 147-153 **(2017)**

6. De Paula Silva P.H., McBride S., De Nys R. and Paul N.A., Integrating filamentous 'green tide' algae into tropical pond-based aquaculture, *Aquaculture*, **284**, 74-80 **(2008)**

7. Dhup S., Kannan D.C. and Dhawan V., Growth, lipid productivity and cellular mechanism of lipid accumulation in microalgae *Monoraphidium* sp. following different phosphorus concentrations for biofuel production, *Curr. Sci.*, **112(3)**, 539-548 **(2017)**

8. Elenkov I., Stefanov K., Dimitrova-Konaklieva S. and Popov S., Effect of salinity on lipid composition of *Cladophora vagabunda*, *Phytochemistry*, **42**, 39-44 **(1996)**

9. Kaewkannetra P., Enmak P. and Chiu T.Y., The effect of CO_2 and salinity on the cultivation of *Scenedesmus obliquus* for biodiesel production, *Biotechnol. Bioproc. E.*, **17**, 591-597 **(2012)**

10. Kakarla R., Choi J.W., Yun J.H., Kim B.H., Heo J., Lee S., Cho D.H., Ramanan R. and Kim H.S., Application of high-salinity stress for enhancing the lipid productivity of *Chlorella sorokiniana* HS1 in a two-phase process, *J. Microbiol.*, **56(1)**, 56-64 (**2018**)

11. Kirst G.O., Salinity tolerance of eukaryotic marine algae, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **41**, 21-53 **(1990)**

12. Lawton R.J., de Nys R., Magnusson M.E. and Paul N.A., The effect of salinity on the biomass productivity, protein and lipid composition of a freshwater macroalga, *Algal Res.*, **12**, 213-220 **(2015)**

13. Mohey El-Din S.M., Accumulation of lipids and triglycerides in *Isochrysis galbana* under nutrient stress, *Appl. Biochem. Biotech.*, **189**, 359-373 **(2019)**

14. Rai M.P., Gautom T. and Sharma N., Effect of salinity, pH, light Intensity on growth and lipid production of microalgae for bioenergy application, *OnLine J. Biol. Sci.*, **15(4)**, 260-267 **(2015)**

15. Satpati G.G. and Pal R., Rapid detection of neutral lipid in green microalgae by flow cytometry in combination with Nile red staining- an improved technique, *Ann. Microbiol.*, **65**, 937-949 **(2015)**

16. Satpati G.G., Mallick, S.K. and Pal R., An alternative high-throughput staining method for detection of neutral lipids in green microalgae for biodiesel applications, *Biotechnol. Bioproc. E.*, **20**, 1044-1055 **(2015)**

17. Satpati G.G., Kanjilal S., Prasad R.B.N. and Pal R., Rapid accumulation of total lipid in *Rhizoclonium africanum* Kutzing as biodiesel feedstock under nutrient limitations and the associated changes at cellular level, *Int. J. Microbiol.*, **275035**, 1-13 **(2015)**

18. Satpati G.G., Gorain P.C., Paul I. and Pal R., An integrated salinity-driven workflow for rapid lipid enhancement in green microalgae for biodiesel application, *RSC Adv.*, **6**, 112340 **(2016)**

19. Satpati G.G., Gorain P.C. and Pal R., Efficacy of EDTA and phosphorus on biomass yield and total lipid accumulation in two green microalgae with special emphasis on neutral lipid detection by flow cytometry, *Adv. Biol.*, **8712470**, 1-12 **(2016)**

20. Satpati G.G. and Pal R., Microalgae- biomass to biodiesel: a review, *J. Algal Biomass Utln.*, **9(4)**, 11-37 **(2018)**

21. Sonkar S. and Mallick N., An alternative strategy for enhancing lipid accumulation in chlorophycean microalgae for biodiesel production, *J. Appl. Phycol.*, **30**, 2179-2192 **(2018)**

22. Verawaty M., Melwita E., Apsari P. and Wiyahsari M., Cultivation strategy for freshwater macro- and micro-algae as biomass stock for lipid production, *J. Eng. Technol. Sci.*, **49(2)**, 261-274 **(2017)**

23. Wang W., Vinocur B. and Altman A., Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance, *Planta*, **218**, 1-14 **(2003)**

24. Wang H., Zhang Y., Zhou W., Noppol L. and Liu T., Mechanism and enhancement of lipid accumulation in filamentous oleaginous microalgae *Tribonema minus* under heterotrophic condition, *Biotechnol. Biofuels*, **11**, 328 **(2018)**

25. Zhang W., Zhao Y., Cui B., Wang H. and Liu T., Evaluation of filamentous green algae as feedstocks for biofuel production, *Bioresour. Technol.*, **220**, 407-413 **(2016)**

26. Zhila N.O., Kalacheva G.S. and Volova T.G., Effect of salinity on the biochemical composition of the alga *Botryococcus braunii* Kütz IPPAS H-252, *J. Appl. Phycol.*, **23**, 47-52 **(2011)**

27. Zhu L.D., Li Z.H. and Hiltunen E., Strategies for lipid production improvement in microalgae as a biodiesel feedstock, *BioMed Res. Int.*, **8792548**, 1-8 **(2016)**.

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