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Effect of Salinity and Iron Stressed on Growth and Lipid Accumulation In *Skeletonema costatum* for Biodiesel Production

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Abstract

High lipid content from selective algal species is the potential source of biodiesel production. Skeletonema costatum is the most attractive algae species for biodiesel production, with low lipid content. Therefore, research work is focused on increasing the lipid content and growth rate by varying the factors like salt and iron concentration which enhance the biodiesel production from Skeletonema costatum. The Skeletonema costatum was grown in Conway's medium at constant pH and temperature of 7 and 24°C respectively for 12 days of incubation in a batch reactor. NaCl and ferrous sulphate were used as the salt and iron source respectively. Various concentrations of NaCl and ferrous sulphate were 0.1mM, 0.2mM, 0.3Mm, 0.4mM, 0.5mM and 10µM, 20µM, 30µM, 40µM, 50µM respectively.30µM $FeSO_4.7H_2O$ and 0.4 mM NaCl resulted the highest growth rate of 0.25 d^{-1} and 0.32 d^{-1} respectively. Also, maximum lipid content of 65.8 %CDW was found at 0.4mM of NaCl and 48.5%CDW was obtained with 30µM of $FeSO_4.7H_2O$ resulted. Thus, it can be concluded that the presence of NaCl and $FeSO_4.7H_2O$ in the media increases the lipid content of Skeletonema costatum after 12 days of incubation, when comparing with the corresponding controls.

Keywords: Salinity, iron stress, growth rate, lipid content, biodiesel.

Introduction

Increased global energy consumption and utilization of fossil fuel causes its depletion and create environmental problems such as energy crises, greenhouse gas emission (NO_x, CO_2 and SO_x), which causes global warming and climatic change problems[1]. Researchers and scientists are trying to identify an alternative source for the fuel from renewable sources such as vegetable oil, non-edible oil, algae oil etc[2]. Nitrogen, phosphorous and other nutrients present in plants are mainly utilized for photosynthesis, energy storage, respiration and mineral uptake[3]. Compared to plants, microalgae have an induced photosynthetic effect and produces high oil content[4]. Microalgae are considered as the most promising substrate for producing biodiesel, which is said to be third generation fuel. Microalgae are highest producing feedstock for biodiesel production. Most microalgae contain TAG-Triacylglycerol and fatty acids, which produce biodiesel[1]. Microalgae utilized the photosynthesis process in growth system and it can store oil in the form of lipid in the membrane, which is converted into energy sources. 29% of lipid content and 0.145g/l of biomass productivity was enhanced by N-limitation condition[5]. Increasing the concentration of algal biomass and lipid content, which exhibit economic point of algal biodiesel production. Lipid content is known to increase by high concentrations of iron, nitrogen and phosphorous[6]. Low iron concentration resulted in decreased chlorophyll concentration, which leads to low biomass and lipid content. Nana Annan reported that salinity stress and high iron will change the photosynthetic process and it will affect the growth condition[7]. Cultivation of nitrogen deficient medium could increase the lipid production[1]. Yingshen Zhijian et al reported that *Scenedesmus dimorphous* 1.8 g/l urea medium and heterotrophic chlorella protothecoides from 2.4g.l nitrate medium achieved maximum yield of 0.4g/l and 5.89g/l lipid respectively[8]. Nitrogen is easily available and cost effective compared to other factors. In various microalgae, nitrogen plays a vital role in the fatty acids and lipid metabolism[3]. Sanjay reported that environmental parameters and physico-chemical properties of fatty acid from renewable sources play a major role in biodiesel production[9]. Mandal and Mallick et al has studied on the effect of nitrate, thiosulfate and cultivation time on biomass such as VC 2013 and *S.obliquu* by using central composite design[10].

Material and Methods

Algae strain used and adoption of Culture Conditions: Unicellular marine microalgae *Skeletonema costatum was purchased from* CMFRI, Tuticorin. Stock culture was maintained in a temperature of 4°C. Stock culture was kept in a one-liter sterile plastic container. Vessels were used in the culturing of algae was sterile and dried before use. Instead of double distilled water sea water has been filtered and sterilized in a medium using 5-micron filter bag. 500ml of Walney's medium in a 1 liter Erlenmeyer flask was taken and it is sterilized in an autoclave at 121°C for 15 minutes by adjusting the pH to 7 using 1M HCl and 1M NaoH solution. After cooling, the medium was transferred into five different conical flasks containing a stepwise concentration of NaCl in the range of 0.1mM to 0.5mM with constant pH of 7. Sterilized air was

continuously supplied to the culture with two fluorescent tubes with 2000 lux. Constant temperature of 24°C was maintained throughout. Similarly, by varying the stepwise concentration of ferrous sulphate 10µM to 50µM, biomass concentration was measured by taking the OD value everyday throughout the incubation periods.

Determination of biomass concentration and productivity: The final dry weight and initial dry weight of the cells were taken and the productivity of biomass was calculated using the equation given in Niels et al[11]. Dry cell weight was determined by filtering 15ml of algal culture through microfiber filter paper with 4.7mm diameter. The filter paper along with the biomass was dried and after drying, the concentration of biomass was rinsed with double distilled water followed by drying process in an oven at 105°C for 24hrs and weighed using an electronic balance.

$$P_{Biomass}(g.L^{-1}d^{-1}) = \frac{X_t - X_0}{t_t - t_0}$$

Where *X*o - initial dry biomass concentration at time t_o, *X*t - final dry biomass concentration at time t_t

Determination of mean growth rate: Mean growth rate is the measure of doubling or generation of the exponential cell that occurs per unit time in a log phase.

Mean growth rate was calculated by the following formula

$$R \text{ or } \mu\ (d^{-1}) = \frac{\ln {}^{N_t}/_{N_0}}{T_t - T_0}$$

Where: T_o – starting day of exponential phase, Tt - final day of exponential phase, N_0 - Number of cells at the starting of exponential phase, N_t - Number of cells at the end of exponential phase.

Lipid Extraction: Extraction of lipids from *Scenedesmes spp* done by the method of Bligh and Dyer[12]. 50ml of algal solution were harvested by centrifugation at 10,000rpm for 20 min at 4°C. 1ml of wet cells, 1ml of chloroform and 2ml methanol was completely mixed by vortex for 10 minutes.1.25 ml of chloroform was added and vortex for 2 minutes. 1.25ml distilled water added into the vortex mixer and agitated at 1000 rpm for 5 minutes at room temperature. Upper layer was decanted and lower organic layer containing the extracted lipids were transferred into another test tubes. Extraction procedure was repeated again until get pure lipids.

Determination of Lipid Content: Organic phase containing wet lipid solution was weighed in the pre-vial (W1) and evaporate the organic layer in a hot oven at 100°C for 60 min and weighed(W2). Lipid yield (or) lipid content was determined by using the following formula.

$$C_{Lipid} = W_1 - W_2$$

Lipid productivity was calculated by

$$P_{Lipids}\left({}^{g}/_{l}.day\right) = C_{Lipid} \times \frac{DCW}{100}$$

Where: P_{Lipid} - Lipid productivity in g L^{-1} day^{-1}. DCW - Dry cell weight at time T.

Statistical Analysis: The mean difference among the experimental data was measured from three replicates using Analysis of variance (ANOVA).

Results and Discussion

Growth and lipid study was conducted on *Skeletonema costatum* using the parameters such as salinity and iron concentration. Species grown on Conway's medium was sterilized and kept for 16hours light regime and 8hours dark regime and then incubated for 12 days. Growth and lipid yield were calculated for each day. The study results show that contains more intracellular lipids started to accumulate in *Skeletonema costatum* on salinity and iron stress conditions, which increased the biomass concentration. Biomass concentration, biomass production, lipid content, lipid productivity was given in the table-1. With various concentration of NaCl, the growth of *Skeletonema costatum* was showed mean growth rate $0.31d^{-1}$ for 12 days of incubation. Biomass concentration of 0.22g/L of *Skeletenoma costatum* resulted in the maximum growth of $0.39d^{-1}$ and lipid content obtained 65.8 %CDW at 0.4 mM of NaCl as shown in figure-1. Lipid productivity in the range of $100.2g.L^{-1}.d$ corresponds to biomass concentration of $2.91g.L^{-1}$ and biomass productivity of $0.6mg.L^{-1}d^{-1}$. Under the optimum concentration of $FeSO_4.7H_2O$, the microalgal biomass concentration reached a maximum of $1.62g.L^{-1}$ with a biomass productivity of $38.3g.L^{-1}.d^{-1}$ and lipid content of 48.5 % CDW and the corresponding lipid productivity was $21.9gL^{-1}.d^{-1}$ for 12 days of incubation period.

Table-1
Effect of NaCl concentration on biomass and lipid production

Concentration of NaCl(mM)	Biomass concentration g/L	Biomass productivity $gL^{-1}d^{-1}$	Lipid Content %	Lipid Productivity $g.L^{-1}.d^{-1}$
0.1	2.30	0.28	43.5	141
0.2	2.52	0.31	46.1	125
0.3	2.55	0.42	45.02	118
0.4	3.26	0.6	65.2	129.2
0.5	2.91	0.21	51.3	98.2

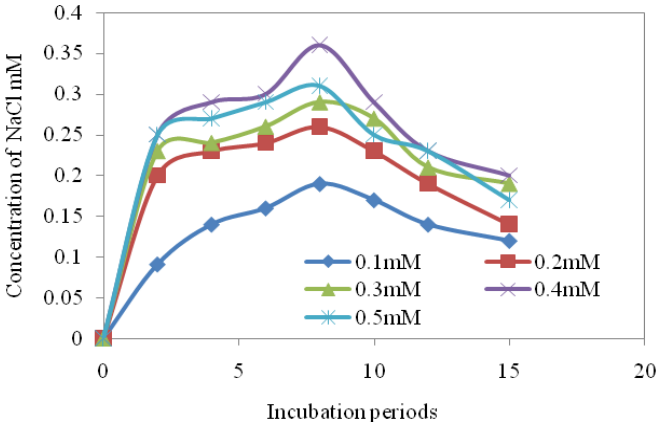

Figure-1
Effect of various concentration of NaCl on growth medium

Discussion: Growth rate of *Skeletonema costatum* in Conway's medium, which gradually increase the multiplication of cells in the all five concentration of NaCl, and the maximum growth rate were measured using the formulae given in Nichols et al., in an incubation time for 12 days[13]. Generation time was found to be in the exponential phase of growth. Reduction in growth rate was observed after the 8th day of incubation at 0.4mM NaCl. Our results agree with Hyder et al., who also found that NaCl concentration inhibited the growth rate of microalgae[14]. The growth of *Skeletonema costatum* at 0.4mM NaCl resulting in increase in the Biomass concentration of 0.6 $g.L^{-1}$ and lipid productivity was 129.2 $g.L^{-1}.d^{-1}$. The maximum lipid content was 65.2% CDW at 8th day of inbition. Lipid content gradually increased upto 8th day after that the culture lipid content was reduced and declined. We concluded that salinity stress of plants and algae are important for osmotic and ionic stress. Water deficit brings about osmotic stress while excess Na^+ and Cl^- reduction in the uptake of their mineral nutrients can brings about ionic balance or stress. Growth of *Skeletonema costatum* with the various concentration of ferrous sulphate is plotted in figure-2 .Addition of 30µM of ferrous sulphate increased the growth rate to $0.25d^{-1}$. Lipid content was 45.5% CDW that inhibit the growth rate with respect to control. Maximum lipid productivity was 21.9 %CDW at µ30M concentration of ferrous sulphate in 12 days old culture. We concluded that iron is very important mineral for photosynthesis and respiration and is needed in the biosynthesis of chlorophyll. Results revealed that the parameters such as cultivation condition, growth rate, lipid and fatty acid composition plays an significant role in maximizing the oil yield for biodiesel production from microalgae *Skeletonema costatum*.

Conclusion

This study provides that the parameters such as salinity, ion concentration is necessary for photosynthesis and respiration of *Skeletonema costatum* can increase the growth and lipid content that induces the great source of biodiesel production. Lipid content was significantly enhanced using 0.4mM Nacl and the maximum lipid content was 65.8 % CDW achieved than ion concentration. Hence, this study concluded that increasing lipid content and growth rate by optimizing the environmental factors will throw a light on improving biodiesel yield.

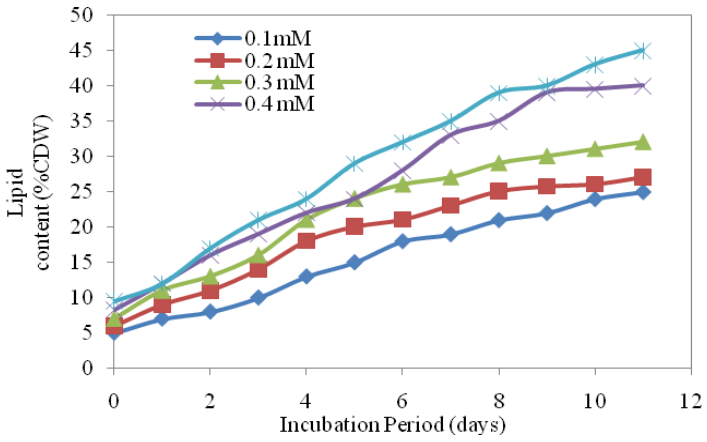

Figure-2
Effect of various concentration of NaCl on Lipid content

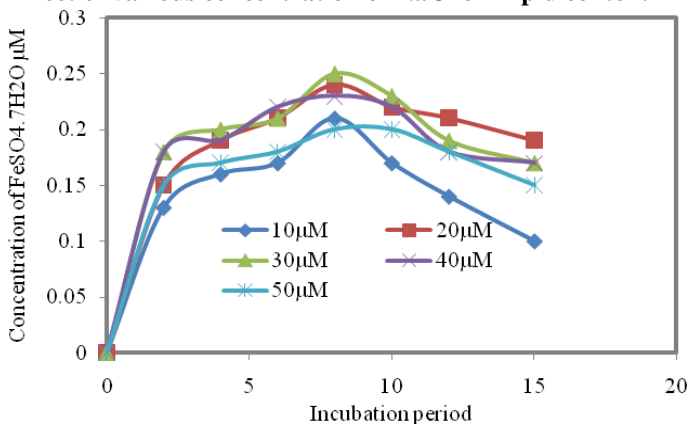

Figure-3
Effect of various concentration of $FeSO_4.7H_2O$ on growth medium

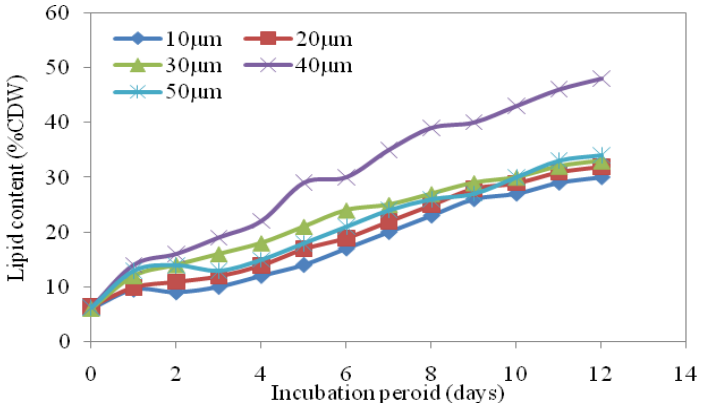

Figure-4
Effect of various concentration of $FeSO_4.7H_2O$ on Lipid content

Table-2
Effect of Ferrous sulphate concentration on biomass and lipid production

Concentration of $FeSO_4.7H_2O$ (µM)	Biomass concentration $g.L^{-1}$	Biomass productivity $g.L^{-1}.d^{-1}$	Lipid Content %	Lipid Productivity $g.\ L^{-1}.d^{-1}$
10	1.59	32.5	45.3	17.2
20	1.62	38.3	48.5	21.9
30	1.04	41.3	39.5	22.3
40	0.98	27.1	32.7	22.3
50	0.84	48.3	29.8	11.4

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