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Biomass Production from Blue Green Microalgae under Nutrient Stress

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Abstract: Recently, Microalga has acquired great potential in many industries such as fine chemicals, pharmaceuticals, and biofuels. The effect of nutrient stress on micro algal growth must be investigated; since it has a major impact on biomass growth and characteristics. The present research investigates two blue green algae species *Microcystis aeruginosa* and *Anabaena constricta* collected from River Nile. Measurements of the chlorophyll a contents, as an indication of the biomass growth rate, in both species are conducted under nitrate starvation and salt stress. Statistical models are derived using response surface technique to describe algae species growth rates. Response surface and contour plots of models' outputs are used to obtain the optimum conditions (time, nitrate and salt concentrations) at which the maximum biomass growth is reached. For *Microcystis aeruginosa*, the investigation shows that the maximum growths are 480 mg/l at 0.8 gm/l $NaNO_3$ and 13 days and 240 mg/l at 13 days under salt stress. With respect to *Anabaena constricta*, the maximum growths are 191mg/l at 0.7 gm/l $NaNO_3$ and 8 days and 240 mg/l at 2.5 gm/l NaCl and 12days.
Key words: biomass, blue green algae, nitrate starvation, salt stress, response surface methodology.

1. Introduction

Microalgae, recognized as one of the oldest living organisms, are thallophytes (plants lacking roots, stems and leaves) that have chlorophyll as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells[1]. Microalgae have been proposed as the potential source for a wide range of products, ranging from fine chemicals and pharmaceuticals to foods and feeds and as a biofuel source. However, only a few are successful on industrial scales due to the high cost associated with the production of microalgae biomass[2]. Recent progress in the production of microalgae has been intensively reviewed[1], and future perspectives have been presented[3].

In recent years, cultivation of microalgae has received renewed attention on account of their utility as a feasible CO_2 sequestration technology[4-6]. Microalgal species upon exposure to sunlight are capable of fixing CO_2 to produce biofuel, osmetics, pharmaceuticals and nutritionaland compounds. These are the miniature sunlight driven biochemical factories and some of the most efficient CO_2 fixers on this planet.

Nitrogen starvation under mixotrophic cultivation resulted in 70% carbohydrates and 25.6% total lipid of Scenedesmus sp., which makes it potential biomass feedstock for bioethanol and biodiesel production[7]. Response surface analysis allowed observing the simultaneous effect of different interactions among important factors promoting lipid accumulation such as the combination of nitrogen limitation and carbon dioxide

concentration, culture time and carbon dioxide, as well as initial nitrate concentration and carbon dioxide concentration[8].

Since the increase in salinity can increase the lipid content of microalgae, but lowers the growth rate of a species. For that object, salinity effect on the growth of microalgae species have to be investigated. The effect of salinity strength, on cells to survive and flourish, was carefully studied[9]. The addition of NaCl promoted the synthesis of free and ester-type xanthophylls as a study of composition and accumulation of stress-induced carotenoids and characterization in the aerial microalgae *Scenedesmus* sp.[10].

The maximum biomass production is required for any algal strain growing process. To achieve this target, it is necessary to model the process. Mathematical Modeling is crucial for developing of mass production facilities in future, instead of the conventional techniques which are time consuming and exorbitant in cost[11-14]. It is suggested that the application of the response surface methodology (RSM) technique provides a studying strategy for the interaction of the parameters using statistical methods.

The present work expresses microalgae growth in blue green microalgal strains *Microcystis aeruginosa* and *Anabaena constricta*, under nutrient stress namely, nitrate starvation and salt stress, in mathematical equations. Hence, the optimum conditions at which maximum growth are determined. The results of this investigation will make the industries based on microalgae biomass become practically feasible.

2. Materials and methods

2.1. Algal isolation and Purification

Algal species were isolated from River Nile water and concentrated via phytoplankton net (80µm mesh size) and using BG11 media for algal isolation[15] Medium, Modified[16]. Algal identification has been done according to the keys of identification[17-18]. Algal strains were isolated by spreading 0.1ml of water samples into petri dishes containing BG11 Medium supplemented with 1.5% agar for solidification. Single colonies of algae were then recultivated in the specified liquid media for each strain as non-axenic batch cultures (50ml) at 25±2°C and 24hr with continuous white fluorescent lamp intensity ≈2500Lux. Some modifications in the nitrate concentration of the BG11 media (1.5 gm/l $NaNO_3$) were made according to different algal species requirements.

2.2. Cultivation of the Isolated Strains

Cultivation was carried out using BG11 media as reported[19], in sterilized 1 liter conical shoulder flasks containing 600ml of the corresponding culture medium under continuous illumination. The cultivation time differed from one strain to another depending on the optimum growth rate till reaching stationary phase which always ranged between (15-20) days. Nutrient Composition of BG11 consistes of Macronutrient (mg/L): $NaNO_3$, 1500; K_2HPO_4, 40; $MgSO_4$, 75($7H_2O$); $CaCl_2$, 36($7H_2O$); Citric Acid, 6.00; Na_2CO_2, 20; Na_2EDTA, 1.00; Ferric Ammonium Citrate, 6.00; Micronutrient (g/l): H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.222; $Na_2\ MoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.079; $Co(NO_3)_2.6H_2O$, 0.0494. Add 1ml/L into the culture medium from the micronutrient. After autoclaving and cooling pH of medium are about 7.1.

2.3. Effect of nitrate concentrations:

The nitrate source used in BG11 is $NaNO_3$ added in concentrations of (0.3, 0.15, 0.075 and 0.0 g/L). The selected isolates were cultivated in BG11 media with 1.5 g/L $NaNO_3$ concentrations until reaching to stationary phase then take equal samples and transferred to media with lower concentration for 15-20 days as adaptation period, then the isolates were sub cultured in these concentration for the experiment until stationary phase and the culture was collected to determine the effect of decreasing $NaNO_3$ concentrations on biomass production.

2.4. Effect of salt stress

The selected isolates were cultivated in BG11 media with salt free until reaching to stationary phase then take equal samples and transferred to media with different concentrations of NaCl (0.5, 1, 2.5 and 5 g/L) and each one sub cultured in each concentration for 15-20 days as adaptation period, then the isolates were sub cultured again for the experiment and harvested at stationary phase to study the effect of salt stress on the biomass production.

2.5. Statistical analysis

The experimental data of produced biomass by following the above procedures were analyzed by response surface methodology[20] using the following second order polynomial equation:

$$Y=\beta_0+\sum_{i=1}^{2}\beta_i X_i+\sum_{ii}^{2}\beta_{ii}X_i^2+\sum_{i=1}^{n}\sum_{j>1}^{n}\beta_{ij}X_iX_j+\sum_{i=1}^{n}\sum_{j>1}^{n}\beta_{ij}X_i^2X_j+\varepsilon$$

where Y is the response (chlorophyll-a) xi and x_j are the coded independent variables and β_0, β_i, β_{ii} and β_{ij} are intercept, linear, quadratic and interaction constant coefficients, respectively and ε is the error. The experimental parameters time and NaNO3 and NaCl concentrations (X_i and X_j) are between -1 and 1 based on the minimum and maximum values of the variable as shown in Table 1 and Table 2. MATLAB R2012a program was used for developing mathematical models' equations for chlorophyll –a content of each species. The quality of the developed models can be determined from the value of correlation (R squared); adjusted statistic coefficient (R^2_{adj}); ratio of variance, computed value (F-test); statistical estimator (p-value). The p-value (probability of error value) is a tool used to determine the significance of each regression coefficient as well as interaction effect of each cross product. The smaller the p-value, the bigger the significance of the corresponding coefficient is. In this case, the p-values less than 0.05 indicated that the particular term was statistically significant[21].

Table: 1 Independent variables and levels for both species under nitrate starvation

Symbol	coded	levels	
		-1	+1
Time (days)	X1	0.0	18.0
$NaNO_3$ (gm/l)	X2	0.0	1.5

Table: 2 Independent variables and levels for both species under salt stress

Symbol	coded	levels	
		-1	+1
Time (days)	X1	0.0	15.0
NaCl (gm/l)	X3	0.0	5.0

The response surface and contour plots are developed using the proposed model equation obtained by least square regression method. Based on the model, the optimum conditions for maximum Chlorophyll-a contents are deduced.

3. Results and discussion

3.1 Algal growth rate

The growth rate of the selected isolates was determined as chlorophyll-a content till reached the Stationary phase under control conditions (BG11 medium, white florescent light with intensity 2500 Lux for illumination period 24hrs), the maximum standing biomasses of *Microcystis aeruginosa* and *Anabaena constricta* were reached after 8 days and continue to grow up to 18 days at these control conditions.

3.2 Development of regression models' equations.

Four models describe the influence of the concentrations of both NaNO3, and NaCl and time on the two blue green algae species chlorophyll-a content using Response Surface Methodology. The chlorophyll-a content is transformed to biomass (mg/l) values by the equation reported[19]. The models that fitted the response (biomass growth) were subject of statistical analysis of variants (ANOVA), to determine the significant individual terms and their interactions and indicated that the models are not aliased. The models equations' based on the coded values (X_1, X_2 and X_3 as time, $NaNO_3$ and NaCl substrates concentrations, respectively) for the responses' (Y_1, Y_2, Y_3 and Y_4) are outlined.

3.3 Statistical analysis of growth models for *Microcystis aeruginosa*

Suggested growth models according to RSM for *Microcystis aeruginosa* are Eq. 1 and Eq. 2 with Y_1 and Y_2 as Chlorophyll-a content for $NaNO_3$ starvation and NaCl stress respectively. According to Analysis of variance (ANOVA), values R squared, adjusted R, *F*-test and *p*-value of the models are presented in Table 3, and these values show that the models fit the experimental data.

$$Y_1 = 3.48 + 1.01\ X_1 + 0.03X_2 + 0.04\ X_1\ X_2 - 0.58X_1^2 - 0.48\ X_2^2 \quad (1)$$

$$Y_2 = 2.66 + 1.06\ X_1 - 0.14X_3 + 0.12\ X_1\ X_3 + 0.03\ X_3^2 \quad (2)$$

The normal probability plots of the residuals examined this analysis and illustrated in Figures 1a and b for *Microcystis aeruginosa* under nitrate starvation and salinity stress respectively. The normal probability plots of the residuals indicate that the errors are distributed normally in a straight line and insignificant. Hence those models adequately represent the experimental data.

Table 3 Models' ANOVA analysis output for *Microcystis aeruginosa* strain under nitrate starvation.

Nutrient	R2	R2 Adj	F-tests	p-value
NaNO3 starvation	0.9394	0.9325	136	< 0.0001
NaCl stress	0.8356	0.8112	34.30	< 0.0001

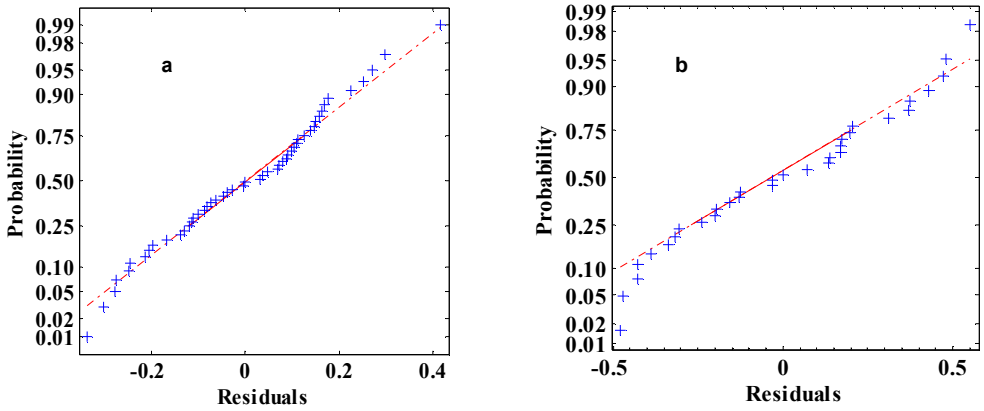

Fig. 1 Normal probability plots for *Microcystis aeruginosa* under nitrate starvation (a) and salinity stress (b)

3.4 Statistical analysis of growth models for *Anabaena constricta*

Suggested models for *Anabaena constricta* under nitrate starvation and salt stress are Eqs. 3 and 4; with Y_3 and Y_4 the responses respectively. Analysis of variance (ANOVA) was further carried out to determine the significance and the fitness of the models. The ANOVA values R squared, adjusted R, *F*-test and *p*-value of the models are presented in Table 4. These values imply that the models are significant.

$$Y_3 = 3.48 + 1.01\ X_1 + 0.03X_2 + 0.04\ X_1\ X_2 - 0.58\ X_1^2 - 0.48\ X_2^2 \quad (3)$$

$$Y_4 = 2.66 + 1.06\ X_1 - 0.14X_3 + 0.12\ X_1\ X_3 + 0.03\ X_3^2 \quad (4)$$

The analysis results illustrate normal probability plots of the residuals in Figures 2a and 2b for *Anabaena constricta* under nitrate starvation and salinity stress respectively. A linear distribution of errors are obtained, indicated that this models provided a good approximation to the experimental data.

Table 4 Models' ANOVA analysis output for Microcystis aeruginosa strain under salt stress.

Nutrient	R2	R2 Adj	F-tests	p-value
NaNO3 starvation	0.9456	0.9362	100.90	< 0.0001
NaCl stress	0.9577	0.9532	211.28	< 0.0001

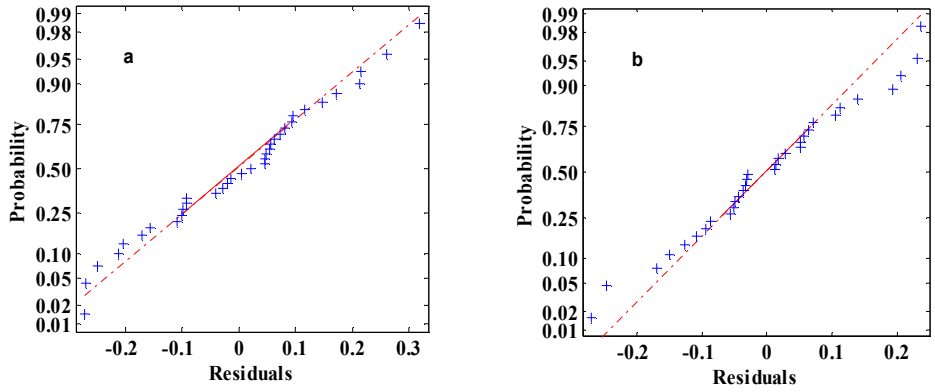

Fig. 2 Normal probability plots for *Anabaena constricta* under nitrate starvation (a) and salinity stress (b).

3.5 The biomass growth of *Microcystis aeruginosa* under substrates stress.

Values of chlorophyll-a content are obtained according to Eqs 1 and 2 as response surfaces and contour plots and presented in Figures 3a and 3b. The contour plot figure 3a showed that the maximum growth is 480 mg/l at 0.8 gm/l $NaNO_3$ and 13 days under nitrate starvation. Figure 3b shows that the salt stress has no tangible effect on the biomass compared to the time. The maximum biomass of the strain under salt stress is 240 mg/l at 13days.

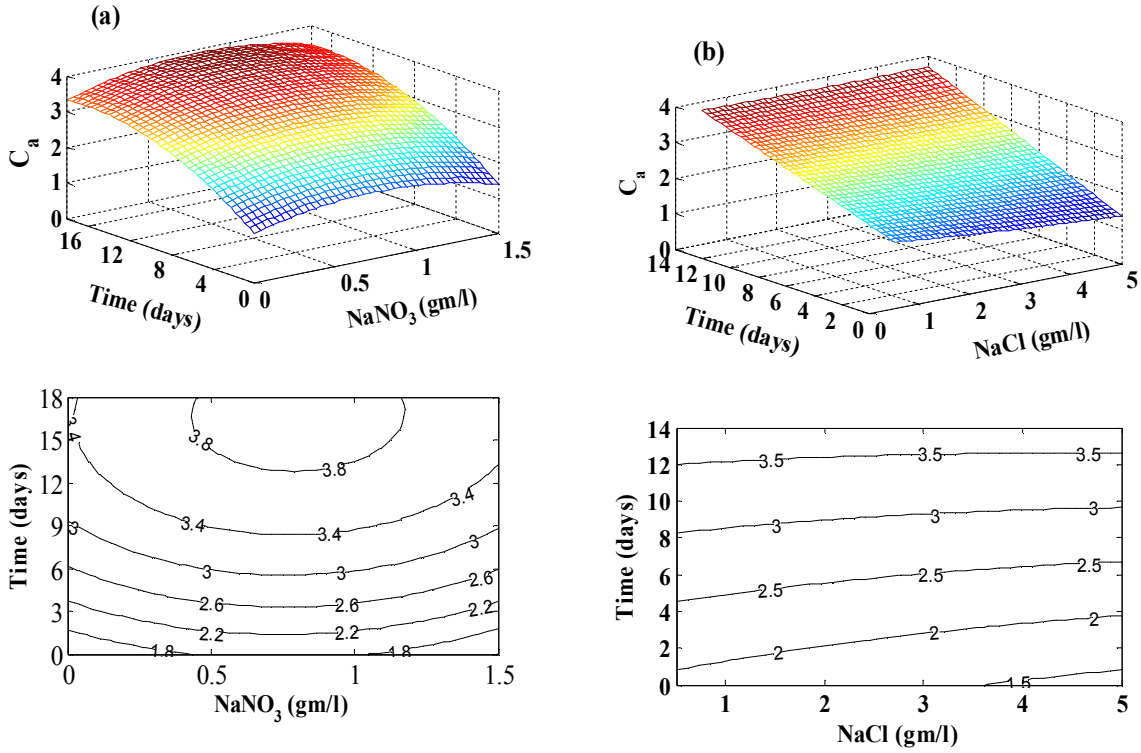

Fig. 3 Response surface and contour plots of biomass production for *Microcystis aeruginosa* under nitrate starvation (a) and salinity stress (b)

3.6 The biomass growth of *Anabaena constricta* under substrates stress

Effect of NaNO3 starvation and NaCl stress on chlorophyll-a content of Anabaena constricta strain is shown in Figure 4a and b. The figures represent the values of chlorophyll-a response surface and contour plots

related to Eq. 3 and Eq. 4. Under Nitrate starvation and salt stress, the maximum biomasses are 191mg/l at 0.7 gm/l NaNO3 and 8 days and 240 mg/l at 2.5 gm/l NaCl and 12days, respectively.

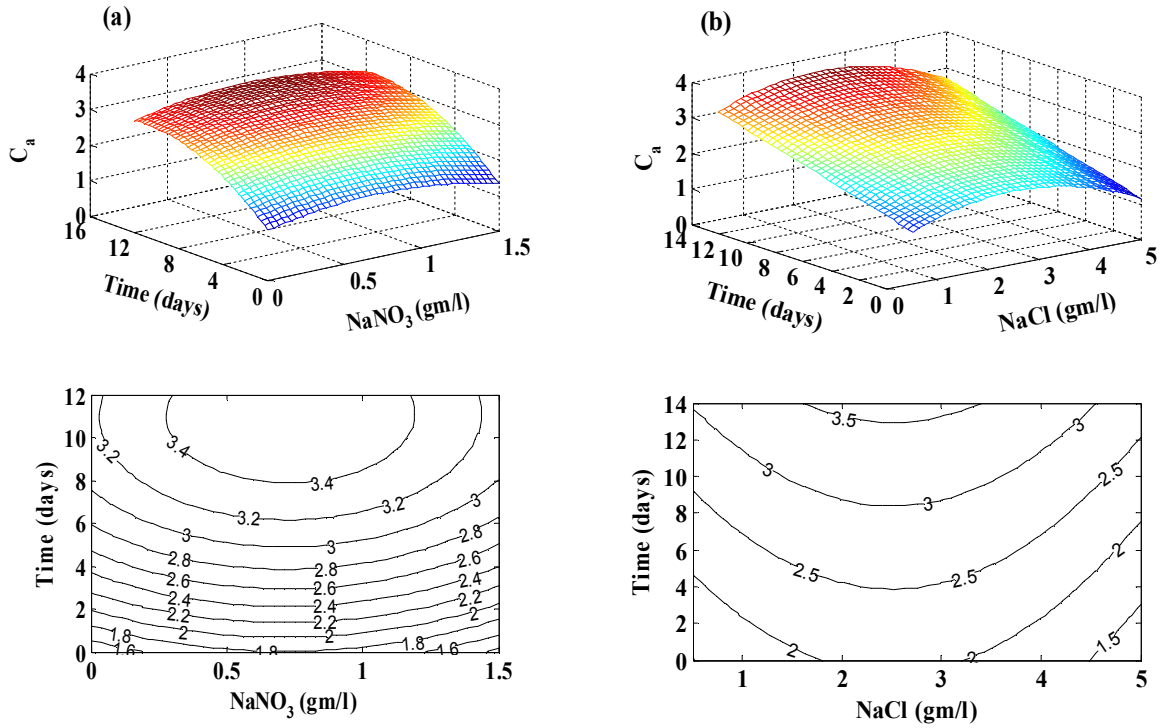

Fig. 4 Response surface and contour plots of biomass production for *Anabaena constricta* under nitrate starvation (a) and salinity stress (b)

4. Conclusion

Mathematical models are developed to describe the effect of niatrate starvation and salt stress on the biomass growth of blue green algae *Microcystis aeruginosa* and *Anabaena constricta*. For *Microcystis aeruginosa*, the maximum growths are 480 mg/l at 0.8 gm/l $NaNO_3$ and 13 days under nitrate starvation and 240 mg/l at 13days under salt stress. For *Anabaena constricta*, the maximum growths are 191mg/l at 0.7 gm/l $NaNO_3$ and 8 days and 240 mg/l at 2.5 gm/l NaCl and 12 days.

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