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Predictive modeling of biomass production by *Spirulina platensis* as function of nitrate and NaCl concentrations

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ABSTRACT

Effects of nitrate (2.0, 2.5, and 3.0 g L^{-1}) and salt (0.5, 1.0, 1.5, 2.0 g L^{-1}) concentrations on biomass production by *Spirulina platensis* was examined in the Schlösser medium. The highest ($p < 0.001$) biomass yields and chlorophyll *a* content was observed at 2.5 g L^{-1} nitrate and 1.5 g L^{-1} NaCl as 3.495 g L^{-1} and 29.92 mg L^{-1}, respectively. Increment rate of biomass production was especially found between 72 and 216 h. Modified Richards, Schnute, Logistic and Gompertz models was successfully predicted ($r^2 > 0.96$ and RSS $\geqslant 0.003$) biomass production by *S. platensis* as function of nitrate and salt concentrations. Low residual sum of squares (RSS) and high regression coefficients (r^2) indicated that used models were well fitted to the experiment data and it could be regarded as sufficient to describe biomass production of *Spirulina* sp. Biological variables i.e. production rate (μ) and lag time (λ) for *S. platensis* ranged 0.012–0.034 h^{-1} and 2.43–5.85 h, respectively from biomass production were successfully predicted by modified Logistic model according to low RSS and *F*-testing value.

1. Introduction

Spirulina sp. is planktonic photosynthetic filamentous cyanobacterium (Vonshak et al., 1982), identified by the main morphological feature of the genus, i.e. the arrangement of multicellular cylindrical trichomes in a helix along the entire length of the filaments. Native people harvested biomass of *Spirulina* from Chad Lake (Africa) and Texcoco Lake (Mexico) as a source of food for centuries (Vonshak, 1997). However, *Spirulina platensis* has been commercially cultivated due to its biotechnological importance since 1970s (Vonshak, 1997). It is known that *Spirulina* is a versatile organism due to its high nutritional value such as rich in protein content (Cohen, 1997; Colla et al., 2007), polyunsaturated fatty acids (γ-linoleic acid) (Sajilata et al., 2008), pigments (Rangel-Yagui et al., 2004; Madhyastha and Vatsala, 2007), vitamin and phenolics (Colla et al., 2007; Ogbonda et al., 2007). Additionally, biomass of *Spirulina* has been performed to removal unwanted materials such as excess fertilizer, heavy metals, textile dyes and pesticides from wastewaters (Chojnacka et al., 2005; Solisio et al., 2006; Pane et al., 2008; Lodi et al., 2008). Moreover, microalgae play a key role in natural food chain of aquatic systems, as a food source for herbivores such as larvae of many species of zooplankton, mollusks, crustaceans, and fishes (Lavens and Sorgeloos, 1996).

Various environmental conditions such as nutrients, light intensity, and pH directly affect the growth of organisms (Danesi et al., 2004; Colla et al., 2007; Ogbonda et al., 2007). Modeling of organisms provides that understanding of its behavior under growth conditions such as temperature, pH, nutrients etc. (Zwietering et al., 1990; Whiting, 1995; Çelekli et al., 2008). Results of models revealed prediction of microbial development, optimization of growth conditions, biovolume and biomass productions, and also estimation of microbial safety and quality in different environmental conditions. Within the last decades, several growth models (Costa et al., 2002; Çelekli et al., 2008) have been used to predict biomass and biovolume productions by microalgae during the cultivation. Growth curves of bacteria have significantly described by predicting models (Zwietering et al., 1990; Bozkurt and Erkmen, 2001). Several mathematical models such as Gompertz, Logistic, Richards, Schnute, and Stannard have been developed to describe the whole microbial growth curve (Zwietering et al., 1990; Whiting, 1995). Sigmoidal growth curve contain mathematical parameters (a, b, c, ...) rather than parameters with a biological meaning (A, μ, and λ) are described by most of the equations. Three parameters models such as modified Gompertz and Logistic models are among the widely used models which give biological parameters such as lag time (λ), specific growth rate (μ), and asymptotic value (A) (Zwietering et al., 1990; Bozkurt and Erkmen, 2001; Çelekli et al., 2008).

Environmental factors closely affect algal growth due to their physiological requirements. This reason it is important to determine the optimum culture conditions for the achievement of high yields of microalgae in standard media (Voltolina et al., 2005; Ogbonda

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et al., 2007). Biomass production by *Spirulina* species are changed by variation in the culture factors such as nitrogen source (Danesi et al., 2002; Colla et al., 2007), phosphate concentrations (Çelekli et al., 2008), initial biomass concentration (Costa et al., 2002), pH and light intensity (Vonshak et al., 1982; Ogbonda et al., 2007). Nitrogen and NaCl are among the environmental factors that play a significant role on the biomass production of *Spirulina* species (Vonshak et al., 1982; Richmond, 1992). It is known that change in environmental factors such as nitrate, phosphate, salt concentrations, temperature, etc. affected biomass production (Zeng and Vonshak, 1998). Presence of high salt in the medium affected photosystem I and II of *Spirulina* species due to its damage effect on protein degradation (Shipton and Barber, 1994). Source of nitrate is needed to produce organic molecules such as protein and carbohydrate. Consequently, nitrate and sodium chloride are chosen to evaluate the effect of these factors on biomass production by *S. platensis*. The objective of the study was (i) to examine the effect of nitrate and salt concentrations on the biomass production by *S. platensis*, (ii) to predict the biomass production by using modified equations of Gompertz, Logistic, Richards, and Schnute; (iii) to determine the best model which describes the curve of biomass production. Besides, relationship between predict variable (algal biomass and biological parameters) and response factors (nitrate and salt concentrations) was evaluated and understand in batch culture.

2. Methods

2.1. Microorganism and growth conditions

The cyanobacterium used in the study, *S. platensis* obtained from University of Ege, EBILTEM Culture Collection, was inoculated on the Schlösser's medium (Schlösser, 1982). Cells were maintained in the culture medium of Schlösser, having the following composition (per liter): 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4 \cdot 7H_2O$, 0.04 g $CaCl_2 \cdot 2H_2O$. All nutrients were dissolved in distilled water containing (per liter): 6 mL of metal solution (97 mg $FeCl_3 \cdot 6H_2O$, 41 mg $MnCl_2 \cdot 4H_2O$, 5 mg $ZnCl_2$, 2 mg $CoCl_2 \cdot 6H_2O$, 4 mg $Na_2MoO_4 \cdot 2H_2O$), 1 mL of micronutrient solution (50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4 \cdot 5H_2O$, 44.0 mg $ZnSO_4 \cdot 7H_2O$, 20.0 mg $CoCl_2 \cdot 6\ H_2O$, 12.6 mg $MnCl_2 \cdot 4H_2O$, 12.6 mg $Na_2MoO_4 \cdot 2H_2O$) and 0.15 mg of B12 vitamin. The culture was incubated under 2.0 klux with measuring light meter (Lutron Lx-130 model) continuous illumination using cool, white fluorescent lamps.

Batch cultivations were carried out in 250 mL erlenmayer flasks containing 100 mL the medium, placed on an orbital shaker at 90 rpm for 240 h. Algal developments were postulated by changes in the concentrations of $NaNO_3$ (N = 2.0, 2.5, and 3.0 g L^{-1}) and NaCl (0.5, 1.0, 1.5 and 2.0 g L^{-1}) (Table 1) at 30 ± 2 °C. Each batch culture was inoculated with an initial *Spirulina* biomass concentration (g L^{-1} dry weight) of 0.2 g L^{-1}, previously adapted to concentrations of nitrate and sodium chloride. Control medium was prepared without cyanobacterium in Schlösser's medium. Experiments were carried out triplicate.

As reported in previous studies (Zeng and Vonshak, 1998; Costa et al., 2002; Colla et al., 2007), *Spirulina* biomass value was calculated through Optical Density (OD) measurements using a spectrophotometer (UV/VIS Jenway 6305) and a calibration curve of OD against dry weight (g L^{-1}) of *Spirulina* biomass. During OD measurement, the tendency of clumping was prevented via using dilution technique for dense culture. During incubation, biomass values were measured for 0.0, 0.5, 1.0, 24, 48, 96, 120, 144, 168, 192, 216, and 240 h. Besides, the effluent was separated from the biomass with acetate membrane filters (0.45 μm pore size, Sartorious, Germany) and the filtrate was waited at 80 °C for overnight. Amount of chlorophyll *a* was determined by spectrophotometer at 665 nm and 750 nm with using methanol method (Youngman, 1978).

Table 1
Experimental design and growth conditions for salt and nitrate concentrations

Runs	Salt (g L^{-1})	Nitrate (g L^{-1})
S1N1	0.5	2.0
S1N2	0.5	2.5
S1N3	0.5	3.0
S2N1	1.0	2.0
S2N2	1.0	2.5
S2N3	1.0	3.0
S3N1	1.5	2.0
S3N2	1.5	2.5
S3N3	1.5	3.0
S4N1	2.0	2.0
S4N2	2.0	2.5
S4N3	2.0	3.0

S1N1 indicates 0.5 and 2.0 (g L^{-1}) salt and nitrate concentrations, respectively.

2.2. Statistical analyses

The non-linear modified Gompertz, Logistic, Richards, and Schnute equations (Table 2) were fitted to experimental data for biomass production by *S. platensis* as described by Çelekli et al. (submitted for publication). The fitting procedure was performed using commercial computer software SigmaPlot version 10.0.1 (Systat Sofware, Inc., California, USA) via the Marquardt–Levenberg algorithm. This logarithm is used to minimize the sum of square of differences between experimental and predict data. Besides, the logarithm calculates biological parameters, and provides residual data with Residuals sum of square (RSS) values.

F-test was used to compare predicted data obtained by using models (Zwietering et al., 1990; Bozkurt and Erkmen, 2001). In all cases, RSS values from Richards and Schnute models were same. Thus, in this test, RSS obtained from Richards model was taken as an estimate of the measuring error due to the lowest RSS values. *F*-values were calculated as

$$F = \frac{(RSS_2 - RSS_1)/(DF_2 - DF_1)}{RSS_1/DF_1}$$

Tested against $F_{DF_1}^{DF_2-DF_1}$

where RSS_1 is the RSS from modified Richards model, RSS_2 is the RSS from the three parameter model (modified model of Logistic or Gompertz), DF_1 is the degrees of freedom from modified Richards model and equals n points-4, and DF_2 is the degrees of freedom from three parameter model and equals n points-3.

Calculated biological parameters [μ is growth rate (h^{-1}), λ is lag time (h), and A is asymptote value (biomass g L^{-1})] among factors were also compared using ANOVA using the SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Tukey's honestly significant difference (HSD) multiple range test was also carried out to distinguish examined groups. In order to evaluate the goodness of fitting, the predicted data obtained from using equations (Table 2) was plotted against the experimental data and regression coefficients (r^2) and residuals sum of square (RSS) values between predicted and experimental data were calculated.

3. Results and discussion

The cyanobacterium, *S. platensis* obtained from University of Ege EBILTEM Culture Collection, was used in the study. The species showed slightly spiral, left direction of helix, 7–10 μm width of cylindrical trichome, and 33–48 μm diameter of spiral with pH tol-