



Predictive modeling of biomass production by *Chlorella vulgaris* in a draft-tube airlift photobioreactor

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ABSTRACT

The objective of this study was to investigate the growth rate of *Chlorella vulgaris* for CO₂ biofixation and biomass production. Six mathematical growth models (Logistic, Gompertz, modified Gompertz, Baranyi, Morgan and Richards) were used to evaluate the biomass productivity in continuous processes and to predict the following parameters of cell growth: lag phase duration (λ), maximum specific growth rate (μ_{\max}), and maximum cell concentration (X_{\max}). The low root-mean-square error (RMSE) and high regression coefficients (R^2) indicated that the models employed were well fitted to the experiment data and it could be regarded as enough to describe biomass production. Using statistical and physiological significance criteria, the Baranyi model was considered the most appropriate for quantifying biomass growth. The biological variables of this model are as follows: $\mu_{\max}=0.0309$ h⁻¹, $\lambda=100$ h, and $X_{\max}=1.82$ g/L.

1. Introduction

Greenhouse gas release (CO₂ being its major component) is a major environmental concern and mitigation of its release is becoming increasingly necessary. The use of a fast growing, photosynthetic biological micro-organism such as microalgae can provide such a solution. Microalgae can survive chiefly on nutrients such as sunlight and air (with increased CO₂ levels), which makes it a desirable agent for CO₂ sequestration and removal. The use of *Chlorella vulgaris* is a natural biological solution that is environmentally friendly and may produce useful by-products [1-3]. *Chlorella vulgaris* is a freshwater unicellular species of the Chlorophyta with a fast growth rate relative to other microalgae species [4, 5] and it is also easy to cultivate [6]. It is cultivated as biomass and used in health foods, food supplements, and feed surrogates [7]. As well as consuming CO₂ and producing oxygen like most photosynthetic microorganisms, *Chlorella vulgaris* has high chlorophyll content. Like many algae species, *Chlorella vulgaris* also contains oil that can be used to produce

biodiesel with an average content of 28–32% [8]. The *Chlorella* species exhibit markedly lower growth rates under heterotrophic growth [9, 10] and given the need for an organic carbon source, it may be costly; therefore, it could be considered desirable to grow *Chlorella vulgaris* entirely photoautotrophically using a photobioreactor with an artificial light source when sunlight is not available [6]. Photosynthesis as a biological energy conversion system is a remarkable process from the viewpoint of energy accumulation. It is the most abundant energy-storing and life-supporting process on earth [6]. Photosynthesis is the light mediated conversion of carbon dioxide to organic cell materials. Carbon dioxide is consumed by microalgae and converted to carbohydrate and oxygen using solar energy. The specific growth rate of microorganisms has been shown to be affected by the substrate concentration. The results of various models have shown the prediction of microbial progression, optimization of growth conditions, biovolume and biomass productions as well as the assessment of microbial safety and quality in distinctive environmental conditions. Within the last few decades, several growth

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models [11, 12] have been used to predict biomass and biovolume productions by microalgae during development. The process of growing curves of bacteria has significantly been described by predicting models [13, 14]. Some mathematical models such as Gompertz, Logistic, Richards, Schnute, and Stannard have been advanced to depict the entire microbial growth curve [14, 15]. The Sigmoidal development curve contains mathematical factors (a,b,c,...) rather than parameters with a biological meaning (A, μ , and λ) that are depicted by most of the equations. Three factors models such as the modified Gompertz and Logistic models are among the greatly used models which give biological factors such as lag time (λ), specific growth rate (μ), and asymptotic value (A) [12-14]. Such models can give important insight into the dependence of mass productivity on certain parameters of a photobioreactor. However, these models produce only qualitative information and cannot be extrapolated to other photobioreactor configurations, other strains, or even distinctive cultivation conditions [16]. Anjos et al. [17] proposed a second-order quadratic equation with CO₂ concentration and aeration rate as independent variables to investigate CO₂ biofixation by *C. vulgaris*. Although this work did consider the dissociation of gaseous CO₂ to dissolved carbon, it ignored the influence of light intensity on CO₂ biofixation. Niizawa et al. [18] developed a model to predict the influence of light quality on the photon absorption rate of microalgal cultures, thus relating the influence of light quality on the growth rate. Çelekli and Yavuzatmaca [16] investigated the effects of nitrate and salt concentrations on biomass production by *Spirulina platensis*. According to their predictions by the modified Logistic model, the production rate (μ) and lag time (λ) for *S. platensis* ranged from 0.012–0.034 h⁻¹ and 2.43–5.85 h, respectively. Lacerda et al. [19] evaluated the use of refinery wastewater on microalgae cultivation for CO₂ biofixation and biomass production in a bubble column photobioreactor. They used five mathematical growth models (Logistic, Gompertz, modified Gompertz, Baranyi, and Morgan) to estimate the biomass productivity in continuous processes and to predict the ensuing parameters of cell growth. The results showed that by employing statistical and physiological significance criteria, the modified Gompertz model was considered the most appropriate for quantifying biomass growth. Therefore, the primary goal of this study was the engineering of the separation of CO₂ using the cultivation of the microalgae species *Chlorella vulgaris*; the main objectives of the study were (i) to predict the biomass production by using the equations of Logistic, Gompertz, modified Gompertz, Baranyi, Morgan, and Richards and (ii) to determine the models which describe the curve of biomass production.

2. Materials and methods

2.1. Microorganism and growth conditions

The microalgal strain used in this study was *Chlorella vulgaris* and was obtained from the Biological Resource Center of the Institute of Materials and Energy, Iran. The stock culture was propagated and maintained in synthetic BG-11 medium [20] with the composition shown in Table 1. The incubation conditions employed were 25 °C, a photon flux density of 15 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and a photoperiod of 12 h.

Table 1. Composition of BG-11 medium

Parameter	Value
NaNO ₃ (g/L)	1.50
K ₂ HPO ₄ (g/L)	0.04
MgSO ₄ ·7H ₂ O (mg/L)	75.0
CaCl ₂ ·2H ₂ O (mg/L)	36.0
Citric acid (mg/L)	6.0
Fe ammonium citrate (mg/L)	6.0
Na ₂ CO ₃ (mg/L)	20.0
Na-EDTA (mg/L)	1.0
H ₃ BO ₃ (mg/L)	2.86
MnCl ₂ ·4H ₂ O (mg/L)	1.81
ZnSO ₄ ·7H ₂ O (mg/L)	0.22
Na ₂ MoO ₄ ·2H ₂ O (mg/L)	0.39
CuSO ₄ ·5H ₂ O (μg/L)	79.0
Co(NO ₃) ₂ ·6H ₂ O (μg/L)	49.4
pH	8

2.2. Photobioreactor design

The measurements were made in a draft-tube airlift photobioreactor (Figure 1). The system was made of 3.3 mm thick, transparent plexiglas, except for the lower 0.25 m regions that were made of stainless steel. This vessel had a 15 cm internal diameter, a draft-tube internal diameter of 10 cm, a height of 2 m, a riser-to-downcomer cross sectional area ratio of 0.8, and a nominal working volume of 20.0 L. The draft-tube was located 4 cm from the bottom of the reactor. The fluid was mixed by sparging with air through perforated sparger pipes (18 holes of 1mm diameter) (Figure 1). The reactor was continuously illuminated with sixteen 20 W fluorescent lamps connected in parallel, located in a photoperiod chamber. The different numbers of lamps on each lateral side of the photoperiod compartment were combined to give the desired light intensity. The airflow into the photobioreactor was supplied

$$y = \frac{C t^v}{K^v + t^v} \quad (6)$$

where C is the asymptotic ln of growth that occurs as t increases indefinitely, t is the time (h), K is the time at which half maximum growth is achieved (h), and v is the curvature parameter. Furthermore, the Richards equation describes the growth of microbial populations as a function of initial population density, time, growth rate, final population density; lag phase duration, and specific growth rate [14]. On this basis, the Richards expression became

$$y = \left\{ 1 + v \exp(1 + v) \exp\left[\frac{\mu}{A}(1 + v) \left(1 - \frac{1}{v}\right) (\lambda - t)\right] \right\}^{(-1/v)} \quad (7)$$

where A is the asymptotic ln X_t/X_0 as t decreases indefinitely, t is the time (h), μ is the specific growth rate (h^{-1}), λ is the lag phase duration (h), X_t is the cell concentration at time t (g/L), X_0 is the initial cell concentration (g/L), and v is the curvature parameter.

The doubling time (t_d) was calculated by using

$$t_d \left(\ln 2 / \mu \right) \quad (8)$$

where t_d is the doubling time (h) and μ is specific growth rate (h^{-1}). To estimate the parameters of the models, the Levenburg–Marquardt (LM) algorithm is still very important. A non-linear regression algorithm of LM was utilized to fit the rival equation expressions to the experimental results by minimizing the summation of the squares of the differences and estimation of the biological parameters (A, B, C, M, K, λ , μ , and t_d) and curvature parameter. The indices of the performance for the predictive models were investigated by the following mathematical and statistical equations as a function of model-predicted values (pred), experimental studied values (obs), mean of observed values (mean obs), and number of samples (n) [29].

Root mean square error (RMSE):

$$RSME = \sqrt{\frac{\sum (obs - pred)^2}{n}} \quad (9)$$

Standard error of prediction (%SEP):

$$\%SEP = \frac{100}{mean\ obs} \sqrt{\frac{\sum (obs - pred)^2}{n}} \quad (10)$$

Bias factor (B_f):

$$B_f = 10^{\frac{\sum \log(pred/obs)}{n}} \quad (11)$$

Accuracy factor (A_f):

$$A_f = 10^{\frac{\sum |\log(pred/obs)|}{n}} \quad (12)$$

3. Results and discussion

3.1. Predictive modeling

Mathematical modeling has been used extensively to calculate cell growth through the estimation of the specific growth rate, duration of the lag phase and cell concentrations that are all needed in the study of microbial growth, and for use in industrial microbiology [12, 16]. The fit of the mathematical models to the optimized condition is shown in Figure 2. The analysis of the data shown was an unsatisfactory fit of the logistic, Gompertz, modified Gompertz, and Richards models to the experimental data. These models did not describe with accuracy the data obtained for *Chlorella vulgaris*. The Baranyi and Morgan models showed a good fit with the experimental data, so the goodness-of-fit or credibility of growth models needed mathematical evaluation before being put into practice [30]. The performance indices of the models tested are presented in Table 2. The RMSE provided an evaluation of the agreement between experimental data and the model. The best model agreement will have a decreased value of RMSE. The lowest values were seen with the Baranyi and Morgan models (0.160 and 0.111, respectively). The bias factor (B_f) gave an impartial indication of the best performance of the model. Ross [29] suggested that perfect concurrence between predictions and observations was represented by a B_f of 1. Higher or lower values indicated a systematic over or undervaluing of the observed values, respectively. The models describing the growth rate with B_f in the range of 0.973–1.006 could be considered good, the range of 0.7–0.9 or 1.06–1.15 was acceptable, and 0.7 or 1.15 was undesirable. Again, the investigated values showed that the Baranyi and Morgan models (0.973 and 0.997, respectively) presented the best fit. The accuracy factor showed the difference between the mean observed values and the predicted values. Increasing this factor resulted in a low capacity for the prediction of correctness between the estimated and true values. The values given by the Baranyi and Morgan models were closer to 1 (1.059 and 1.030, respectively). The standard error of prediction (%SEP) approved the lower residuals of the Baranyi and Morgan models. Statistical analysis of the models showed that the Baranyi and Morgan models were the best equations to describe data on the growth of *Chlorella vulgaris*. Thus, it was necessary to evaluate the characteristics of each model to select the most fitting for the prediction. The selection criterion was the significance of model parameters [31]. The Baranyi model had parameters with physical significance (μ , λ , and X_{max}) and the Morgan model was related to the curve fit and can't be physically interpreted. The Baranyi model more closely matched empirical data than other sigmoid functions data, both in terms of statistical accuracy and ease of use [32]. Therefore, the Baranyi model was selected to predict the growth of *Chlorella vulgaris* and was considered statistically

sufficient and robust enough to depict the data on the growth of the tested organism. The Baranyi model gives the following values for the growth parameters of the culture medium: $\mu_{\max}=0.0309 \text{ h}^{-1}$, $\lambda=-100 \text{ h}$ and $X_{\max}=1.82 \text{ g/L}$. The maximum biomass concentration of *C. vulgaris* obtained in this work was $1.82 \pm 0.11 \text{ g/L}$ for an incident light intensity of 1300 lux, and 4% (v/v) CO_2 after 168 hours of incubation. A difference of 5.2% between the values of μ_{\max} calculated with the mathematical growth model and those obtained from the graph of $(\ln X)$ vs. (t) was observed. A negative lag phase was obtained for the alga in the Baranyi model. It was concluded that the acclimated algal culture was conveyed to the medium, which caused easy modification to environmental conditions. Indeed, Hodaifa et al. [33] remarked that the growth curve of *S. obliquus* showed no lag phases for all the experiments. The first phase was exponential growth during cultivations. In addition, Masson et al. [34] noticed that the negative signs of the lag time referred to no modification for microbial growth at environmental conditions. This value of the specific growth rate was in good agreement with Jacob-Lopes et al. [35] who reported a specific growth rate of 0.03 h^{-1} for a *Aphanethece microscopica* Nägeli strain grown at 30°C with a light intensity of 11 klux and 25% (v/v) CO_2 in the feed gas. However, several studies have found lower values for *C. vulgaris*: Greque de Morais and Costa [36] reported a value of 0.010417 h^{-1} for *C. vulgaris* grown with 6% or 12% (v/v) CO_2 , a light intensity of $43 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and at 30°C . Chiu et al. [37] found a specific growth rate of 0.0125 h^{-1} at 5% (v/v) CO_2 , 26°C , and $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ light intensity. In another

work, Sinetova et al. [38] studied *Cyanothece* sp. ATCC 51142 growth under different irradiance and they concluded that the specific growth rate for this cyanobacterium, in the exponential phase of the culture, was not dependent on light irradiance. On the contrary, they showed that the photosynthetic activity increased with light irradiance. Hulatt et al. [39] found a maximal biomass concentration of $3.79 \pm 0.05 \text{ g/L}$ with *C. vulgaris* and $3.60 \pm 0.74 \text{ g/L}$ with *Dunaliella tertiolecta* ($350 \mu\text{mol m}^{-2} \text{ s}^{-1}$ light and 4% v/v CO_2 for both algal species). Ho et al. [40] presented a maximal biomass concentration of 3.51 g/L for *Scenedesmus obliquus* ($160 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 10% v/v CO_2). Nevertheless, Chiu et al. [37] reported a maximal biomass concentration of 1.4 g/L for *C. vulgaris* cultivated at 2% (v/v) CO_2 and a light intensity of $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The higher light intensity used in this last work could have led to photo inhibition of the culture, explaining the smaller biomass productivity compared with other results [37]. But these comparisons must be approached cautious due to the strong dependence of growth on many parameters: temperature, pH [8], medium composition [41], gas flow rate [42] and bioreactor design. CO_2 biofixation by microalgae is related to the growth of algal cells [17, 43] and some desired products are accumulated during the exponential growth stage (e.g., DHA, astaxanthin, etc.) [44, 45]. Thus, a thorough understanding of the time required for microalgae to reach the stationary phase can help to optimize the CO_2 biofixation rate and guide the timing of harvest for useful products.

Table 2. Predicted biological and curvature parameters (A, B, C, M, K, λ , μ , v and td) and Data on statistical model validation from the primary models

	Logistic	Gompertz	Modified Gompertz	Baranyi	Morgan	Richards
A	2.761	-39.953				5.758
B	0.041	0.022				
C	2.761	45.693	5.603	5.710	10.212	
M (h)	23.60	-99.99				
K (h)					101.99	
λ (h)			-20.925	-100		-10.202
μ (h^{-1})			0.0641	0.0309		4.708
v					0.541	-0.973
td (h)			10.813	22.43		
RMSE	0.377	0.274	0.333	0.160	0.111	0.272
B_f	1.006	0.993	1.0009	0.973	0.997	0.993
A_f	1.098	1.072	1.086	1.0588	1.030	1.071
%SEP	9.81	7.136	8.670	4.184	2.899	7.100
R^2	0.941	0.969	0.954	0.989	0.994	0.969

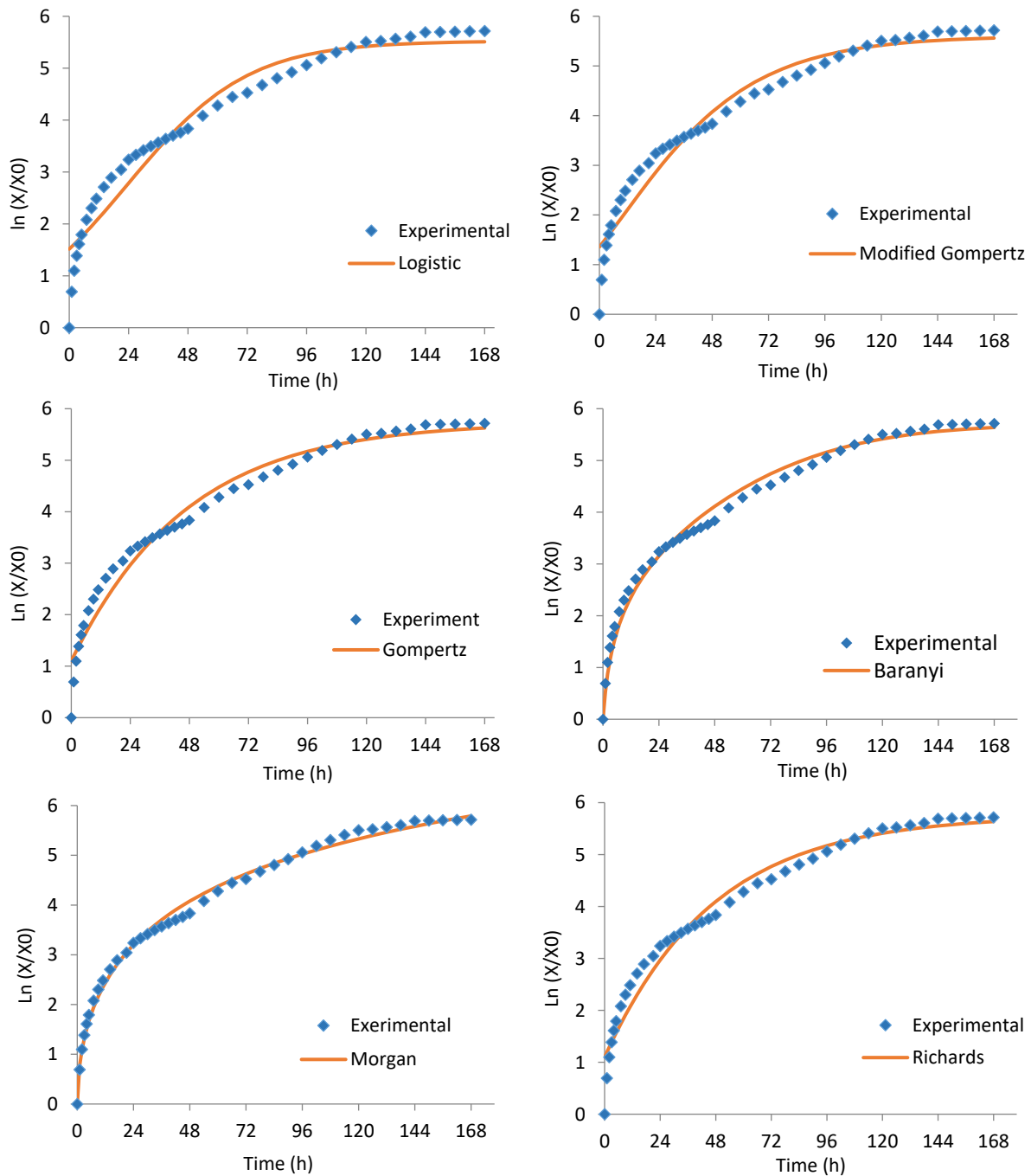


Fig. 2. Growth of *Chlorella vulgaris* in B-11 culture medium. Continuous lines: predicted growth curves were obtained from logistic, Gompertz, modified Gompertz, Baranyi, Morgan and Richards models.

4. Conclusions

Chlorella vulgaris is a freshwater unicellular species of the Chlorophyta which grows rapidly and is easily cultivated. In this study, the mathematical growth modeling of *Chlorella vulgaris* and the prediction of biomass productivity in a draft-tube airlift photobioreactor were investigated. The Baranyi and Morgan models were significantly better than the logistic, Gompertz, modified Gompertz, and Richards models to predict the biomass production of the species.

The Baranyi model more closely matched the experimental data than the other sigmoid functions, both in terms of statistical accuracy and ease of use. The predicted data obtained from the model expressed maximum specific growth rates of 0.0309 h^{-1} and a maximum cell concentration of 1.82 g/L .

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