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# Coupling Non-Linear Regression Analysis with a Predictive Model Using Spectrophotometric Data to Estimate Microalgae Concentration

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**Abstract**

The estimation of algal biomass requires monitoring the growth of microalgae. In contrast to time-consuming methods such as cell counting, spectrophotometry was developed as a straightforward, quick, and explicit method to measure biomass concentration. Non-linear models can appropriately describe the patterns of growth and product formation, which are necessary for any biotechnological process using microorganisms. This study investigated the relationship between algal concentration and absorbance in the 600-750 nm wavelength range. Four mathematical growth non-linear models were utilized to analyze and confirm growth curve-based absorbance data obtained from *Chlorella sorokiniana* and *Chlorella sp.* The calibration curve was then created by relating the absorbance value (680 nm) with the cell density and dry weight measurements and calculating the correlation coefficient. Then, the absorbance derivative was estimated to improve the algal concentration detection limit. A prediction model was created that considered the application of spectrophotometry data to the growth of *Chlorella sorokiniana* and *Chlorella* sp. The Exponential Plateau model was selected to describe the growth of both *Chlorella sorokiniana* and *Chlorella* sp. The significance criteria, i.e., high regression coefficients ($R^2$) and low root-mean-square error (RMSE), indicated that the models used fitted the experimental data well, and may be considered sufficient to characterize biomass concentration. In addition, percentile deviation revealed that the equations obtained in this study could be used to estimate densities with an error of less than 5% for up to $10^7$ cells $mL^{-1}$ and a dry weight of 0.02-1.24 in *Chlorella sorokiniana* and 10% for 0.03-1.18 g $L^{-1}$ in *Chlorella sp.* cultures.

## 1. Introduction

There has been rising interest in using marine organisms to create high-value functional foods that are both nutritious and beneficial to human health. Microalgae are the only marine resources that yield a wide range of bioactive chemicals in terms of structure and chemical composition (Cunha & Grenha, 2016). Microalgae have long been used to produce phytochemicals and are now becoming a significant industry (Cunha & Grenha, 2016). In many parts of Asia, marine algae constitute an essential part of the diet. Microalgae (*Nostoc sp.*) were first utilized as

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food in China over 20 centuries ago, and later, Japan, Mexico, and Taiwan began consuming Chlorella sp. and Spirulina sp. as salutary foods in commercial forms (Cunha & Grenha, 2016). Prehistorically, microalgae have also been used in traditional medicine as a source of nutrients (Ale & Meyer, 2013).

Marine microalgae production has the advantage of being easy and requiring only a simple medium containing seawater (a source of nitrogen, phosphate, iron, magnesium, and certain minor minerals) (Cunha & Grenha, 2016).

Although microalgae cultivation has garnered a lot of attention over the past decades, it still faces many challenges in monitoring on a large scale. Regarding the relevance of microalgae, significant efforts are being made to investigate, develop, and commercialize various aspects of monitoring cell microalgae growth (Santos-Ballardo et al., 2015). Currently, dry weight determination and cell count using a hemocytometer are the most popular ways of assessing cell growth. Dry weight determination is a practical but time- and material-consuming technique to evaluate microalgal growth. Direct cell counting, on the other hand, requires time and operator expertise (Baldisserotto et al., 2022). The limited growth rate of microalgae and the time-consuming nature of conventional growth monitoring methods affect the commercial profitability of microalgae cultivation. Consequently, efforts have intensified to develop microalgae biomass measurement methods that are simpler, more precise, and faster (Ambriz-Pérez et al., 2021). For example, optical density (OD) can be used for rapid and reliable indirect measurement of microalgal cell density and biomass concentration that correlates light absorbance with the algal cell density and dry weight at specific wavelengths (Cunha & Grenha, 2016, Schagerl et al., 2022). Alternately, microorganisms' behavior under various chemical and physical variables, such as pH, time, and temperature, may be described using mathematical models (Hanief et al., 2020). Mathematical modelling of microbial growth has been frequently utilized to calculate cell growth. The established microbial growth models have generally been mathematically simulated so that the theory derived from the models is relevant to experimental results. Over the past few decades, several non-linear growth models, such as Gompertz, Richards, modified Richards, Logistic, Exponential plateau, Beta growth, Schnute, and Stannard, have been proposed by researchers to characterize and predict the growth parameters of microalgae (Cunha & Grenha, 2016; Islam et al., 2022). For example, the Gompertz model has generally proven to be a more flexible and accurate way to adapt to growth data compared to other alternative models. The internal dynamic is represented by the logistic growth curve for both homogeneous and heterogeneous cell populations. Moreover, Pruitt and Kamau found each cell population in an exponential model dies at a rate that is directly proportionate to its size at any given time(Pruitt & kamau, 1993). Additionally, the biomass mass balance is described using the logistic equation for the maximum biomass concentration of microalgae.

In this work, the spectrophotometric approach was employed to evaluate the maximum absorbance of two green microalga strains isolated from the Persian Gulf and Qeshm Island of Iran, Chlorella sorokiniana and Chlorella sp. Moreover, four non-linear models, Exponential plateau, Gompertz, Logistic, and Beta growth models, were applied to describe the growth of the green microalgae species. The growth kinetics of both microalgae species were studied, and a regression model was calibrated using spectrophotometric absorbance to estimate cell density (cells mL−1) and dry weight (g L-1), resulting in an efficient, fast, stable, and selective approach for measuring microalgae biomass concentration.

## 1. Material and methods

### 2.1. Growth conditions

*Chlorella sorokiniana* and *Chlorella sp.* were deposited as strains M8011 and M8010 in the Persian Type Culture Collection (PTCC) after being discovered from the seawater in the Persian

Gulf close to Qeshm island (26°32 N, 53°56 E) in southern Iran. They were grown in BBM medium in Erlenmeyer flasks. The cultures were kept at ambient temperature and subjected to an irradiance level of 70 mol photons $m^{-2}s^{-1}$ while being exposed to fluorescent lighting in a cycle of 12: 12 hours of light/ dark, respectively.

### 2.2. Maximum absorbance determination

Maximum absorbance was calculated for each species of microalgae by scanning sample cultures with a UV-Vis spectrophotometer (BioTek, Epoch, Gen5) between the wavelengths of 600 and 750 nanometers. Then, the maximum absorbance value for each microalga was used as the input to generate an optical density (OD) growth curve (Cunha & Grenha, 2016).

### 2.3. Statistical models

Microalgae growth was analyzed using several different non-linear mathematical models, including the Gompertz, Logistic, Exponential plateau, and Beta growth. The Logistic, Gompertz, Beta growth, and Exponential plateau equations used to calculate the growth of *Chlorella sorokiniana* and *Chlorella* sp. during cultivation are shown in Eqs.1, 2, 3, and 4, respectively (Cunha & Grenha, 2016):

$$Y = \frac{A+C}{1+exp-B(t-M)} \quad (1)$$

$$Y = A + Cexp^{exp\,-B(t-M)} \quad (2)$$

where B is the relative growth rate at time M ($day^{-1}$), A is the asymptotic ln $(OD)t/(OD)_0$ as t drops endlessly, and C is the asymptotic ln $(OD)t/(OD)_0$ as t grows endlessly.

$$Y = Ym \times (1 + (Te - X)/(Te - Tm)) \times (X/Te)\hat{}(Te/(Te - Tm)) \quad (1)$$

where $Y_m$ $Ym$ is asymptotic ln $(OD)t/(OD)_0$ at the peak, $Te$ is the time at asymptotic ln $(OD)t/(OD)_0$ as increases, and $Tm$ is the time of growth point.

$$Y = Ym - (Ym - Y0) \times exp(-k \times x) \quad (2)$$

where $Y_0$ is ln $(OD)t/(OD)_0$ at the $t_0$, $Y_m$ ln $(OD)t/(OD)_0$ as t increases indefinitely, and K is the relative growth rate at the time. In general, t is the time (day), M is the time at which the absolute growth rate is at its maximum (day), $OD_t$ is the optical cell density at time t, and $(OD)_0$ is the initial optical cell density.

### 2.4. Cell growth efficiency

Cell growth was tracked and measured three times a day until the culture reached the stationary phase, at which point it was declared dead. Three distinct types of analysis were used as follows: 1. cell density (cells $mL^{-1}$) was measured using a Neubauer chamber and microscope (Olympus CX40, NY, USA). 2. The absorbance of cell suspensions was evaluated by a UV-visible spectrophotometer (BioTek, Epoch, Gen5), determination of the biomass's dry weight at 60°C. 3. Specific growth rate and duplication time were measured using the growth kinetics (Godoy-Hernández & Vázquez-Flota, 2006). Specific growth rate (μ) and duplication time (dt) (Eqs. 5 and 6) or doubling $time^{-1}$ of the microalgae were calculated in the following equation (Adar et al., 2016):

$$\mu(\kappa) = \frac{Ln\left(\frac{ODt}{ODo}\right)}{t2-t1} \quad (3)$$

where $OD_t$ and $OD_0$ are the optical density at times $t_2$ and $t_0$, and k or μ is expressed in $time^{-1}$.

Equation 6 was used for calculating duplication time:

$$dt = \frac{ln2}{\mu} \quad (4)$$

### 2.5. Predictive models

The predictive models were created by applying Eqs. 7, 8, and 9 to determine the relationship

between spectrophotometric absorbance and cell counting, absorbance and dry weight, and cell counting and dry weight, respectively:

$$Abs = \beta_0 + \beta_1(cells\ mL^{-1}) + \beta 2\ (cells\ mL^{-1})^2 \quad (5)$$

$$Abs = \beta_0 + \beta_1(g\ L^{-1}) + \beta_2(g\ L^{-1})^2 \quad (8)$$

$$cells\ mL^{-1} = \beta_0 + \beta_1(g\ L^{-1}) + \beta(g\ L^{-1})^2 \quad (9)$$

The coefficient of determination ($R^2$), adjusted determination coefficient ($R^2$ adj), and root mean square error (RMSE) were evaluated as performance parameters as follows:

$$R^2 = \frac{SSR}{SST} = \frac{\sum_{i=1}^{n}(\hat{y}-\bar{y})^2}{\sum_{i=1}^{n}(y_i-\bar{y})^2} \quad (10)$$

$$R^2_{adj} = 1 - \frac{\frac{SSR}{n-k-1}}{\frac{SST}{n-1}} = 1 - \frac{\Sigma_{i=1}^{n}(\hat{y}-\bar{y})^2/(n-k-1)}{\Sigma_{i=1}^{n}(y_i-y^-)^2/(n-1)} \quad (11)$$

$$rmse = \sqrt{\frac{SSE}{n-k-1}} = \sqrt{\frac{\Sigma_{i=1}^{n}((y_i-\hat{y}_i)^2}{(n-k-1)}} \quad (12)$$

where SSR, SST, and SSE are the sum of the square regression, the sum of the square total, and the sum of the square residual, respectively. $y$, $\hat{y}$ and $\bar{y}$ are the actual value, the predicted value, and the mean of the actual value, respectively, for n samples of algae species, and k is the number of features in a given frequency range (Basak et al., 2021).

Using the following equation, the percentile deviations (PD) of both the obtained and predicted absorbance using the growth rate theory for each microalgae species were determined to ensure the accuracy of the proposed model (Santos-Ballardo et al., 2015).

$$Percentile\ deviation = \left(\frac{Abs-Abspred}{Abs}\right) \times 100 \quad (13)$$

$$Percentile\ deviation = \frac{cell\ count-cell\ count\ pred}{cell\ count} \times 100 \quad (14)$$

$$Percentile\ deviation = \frac{Dry\ Weight-Dry\ Weight\ pred}{Dry\ Weight} \times 100 \quad (15)$$

### 2.6. Statistical Analysis

The experiments that were used for all of the computations were evaluated in triplicate, and the results were shown in Graph Pad Prism 9 as the mean ± SD.

## 3. Results and Discussions

### 3.1 Microalgae maximum absorbance

To determine and compare the growth patterns of microalgae cultures in terms of biomass yield, two microalgae cultures were cultivated in batch culture for up to 28 days in BBM medium culture under room temperature.

The light absorbance of the various microalgae samples was analyzed from 600 to 750 nm (Fig1). The highest absorbance was obtained at 680nm, which corresponded to the wavelength of maximum sensitivity for quantifying two microalgae samples. Maximum absorbance was measured at various cell growth phases and exhibited the same pattern in all tests. For example, the p-value for Tukey's HSD test (p-value <0.0001, $R^2$: 0.996) was much lower than the cutoff for another wavelength of 680 nm. As a result, this wavelength was used for all subsequent studies. Since pigment absorption is more concentrated at specific wavelengths of light, choosing a wavelength in the range of maximum pigment absorption should provide the largest signal (Griffiths et al., 2011). The maximum absorption ranges of chlorophyll-a in previous experiments on cell growth of some species of microalgae have been reported in different wavelengths, including 664 to 678 nm (Cunha & Grenha, 2016), 680 nm (Cunha & Grenha, 2016), and 684 nm (Cunha & Grenha, 2016).Standard spectrophotometric assessment of

microalgae development typically recommends a wavelength range of 664 to 690 nm since these values coincide with chlorophyll absorption (Cunha & Grenha, 2016). Changes in maximal absorption of microalgae species can be attributed to differences in the content of intracellular pigments and chlorophyll ("a" and "b"), such as carotenoids (Bricaud et al., 1998).

The growth rate finding in this study supported previous research that found values of μ: 0.18–0.36$d^{-1}$ for *C. sorokiniana* (Cunha & Grenha, 2016) and values of μ: 0.192 ± 0.021 $d^{-1}$, dt: 3.6 d, for *Chlorella sp.* (Hajjar Rakhmadumila & Setiani Muntalif, 2020). The specific growth rate values μ: 0.25 - 0.27 $d^{-1}$ were reported for *Chlorella vulgaris* (He et al., 2020). The variation of growth rates of various algae strains depends on growth conditions and algal requirements (Santos-Ballardo et al., 2015). It is clear that different strains of microalgae have distinct cell growth characteristics. This can be explained by the fact that a variety of parameters, including culture conditions (aeration, light, temperature, pH, nutrients) and reactor characteristics, affect microalgae cell growth (Guedes & Malcata, 2012)

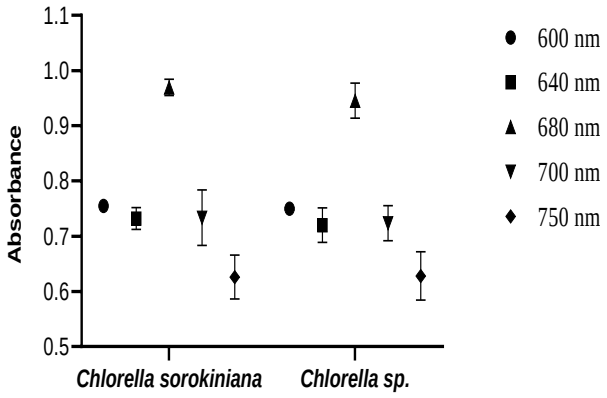


**Figure 1.** The scanning of light absorbance patterns for various strains of microalgae from 600 to 750 nm.

### 3.2 Growth performance of microalgae species

Maximum absorbance was achieved at the end of the stationary phase, as determined by analyzing cell growth curves (Fig 2). Also, to describe the circumstances under which optimal maximum growth is typically seen, the experimental growth period data based on OD were checked, evaluated, and validated for the two species of microalgae using non-linear mathematical models, such as Gompertz, Logistic, Exponential plateau, and Beta growth models. Data from microalgal growth experiments were fitted to a variety of models, as depicted in Figure 2, and all models approximately successfully fit the microalgae growth curves.

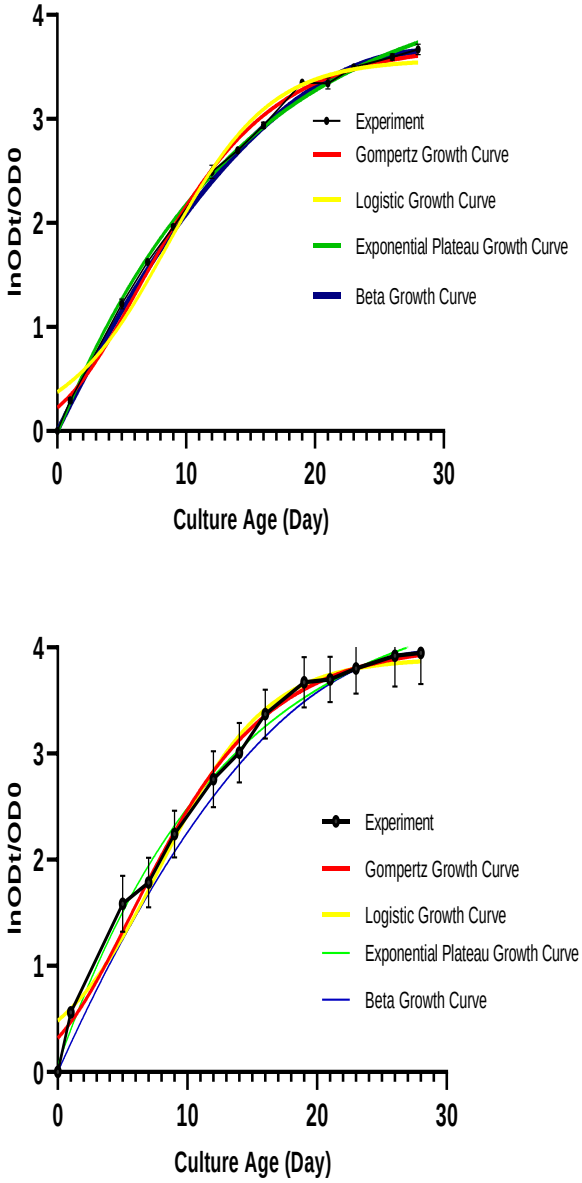


**Figure 2.** Predicted growth curves were obtained from Logistic, Gompertz, Exponential Plateau, and Beta Growth models of a. Chlorella sorokiniana and b. Chlorella sp. in BBM culture medium.

The performance indices data of the models shown in Table 1 indicated that the models provided an acceptable fit to the experimental data. Table 1 also shows that all models explained over 95% of the variation in microalgae optical density, with an $R^2$ value of more than 0.95. Moreover, culture time is a crucial element of the microalgae development profile, and a high adj$R^2$ value indicates that no overfitting has occurred. The decreased value of the root means square error (RMSE) and the Akaike information criterion (AIC) (Table 1) indicated the best agreement between experimental data and the mathematical models.

**Table 1.** Statistical Model Evaluation

| Statistical parameter | *Chlorella sorokiniana* | | | | *Chlorella* sp. | | | |
|---|---|---|---|---|---|---|---|---|
| | Logistic | Gompertz | Exponential plateau | Beta growth | Logistic | Gompertz | Exponential plateau | Beta growth |
| $R^2$ | 0.9826 | 0.9933 | 0.9972 | 0.9972 | 0.9567 | 0.9661 | 0.971 | 0.959 |
| $R^2_{adj}$ | 0.9816 | 0.9929 | 0.9971 | 0.9970 | 0.9542 | 0.9641 | 0.969 | 0.957 |
| RMSE | 0.16 | 0.099 | 0.064 | 0.064 | 0.26 | 0.23 | 0.215 | 0.25 |
| SSM* | 0.98 | 0.37 | 0.156 | 0.157 | 2.58 | 2.017 | 1.72 | 2.40 |
| AICc** | -134.5 | -171.8 | -206.1 | -205.8 | -92.99 | -102.4 | -108.3 | -95.72 |

**Sum of Squares, ** Akaike information criterion*

A mathematical evaluation of the goodness-of-fit or credibility of growth models was necessary before using them to estimate the cell counts based on optical density, even if all models were an excellent match for the experimental data. However, the high determination coefficients ($R^2$ > 0.99 and $R^2$>0.97 for *Chlorella sorokiniana* and *Chlorella* sp., respectively) and high accuracy (RMSE; 6% and 21% for *Chlorella sorokiniana* and *Chlorella* sp., respectively) showed that the experimental results were a good fit for the exponential plateau model. This is enough to explain the growth of the two microalgal strains in BBM medium.

In agreement with the use of mathematical models for microalgae growth monitoring, Lacerda et al. calculated and predicted the parameters of the *Aphanothece microscopica Nägeli (RSMan92)* cell growth using Logistic, Morgan, Gompertz, and modified Gompertz, and Baranyi growth models (2011). Also, Mansouri et al. analyzed and forecasted the biomass production of *Chlorella Vulgaris* using the equations of six mathematical growth models (Logistic, Gompertz, modified Gompertz, Morgan, Richards, and Baranyi), and reported well-fitting models (2016). The Logistic and Gompertz mathematical models were used by Ajala and Alexander to fit and validate the growth period experimental data for *Scenedesmus obliquus*, *Chlorella vulgaris*, and *Oocystis minuta* (2020). They discovered that the Gompertz model fits the microalgae growth curves more closely than the Logistic model for all growth circumstances and microalgae species (Ajala & Alexander, 2020).

The maximum density and absorbance parameters for growth rate and cell growth performance are shown in Table 2. Previous studies on *C. sorokiniana* and *Chlorella* sp. showed values of μ: 0.18–0.36$d^{-1}$ (Rosenberg et al., 2014; Badar et al., 2017; Asadi et al., 2019) and values of μ: 0.192 ± 0.021 $d^{-1}$, dt: 3.6 d, respectively (Hajjar Rakhmadumila & Setiani

Muntalif, 2020) which we confirm with our results. The specific growth rate values μ: 0.25 - 0.27 $d^{-1}$ were reported for *Chlorella vulgaris* (He et al., 2020). The variation of growth rates of various algae strains depends on growth conditions and algal requirements (Santos-Ballardo et al., 2015). It is clear that different strains of microalgae have distinct cell growth characteristics, which can be explained by the fact that a variety of parameters, including culture conditions (aeration, light, temperature, pH, nutrients) and reactor characteristics, affect microalgae cell growth (Guedes & Malcata, 2012). Our results show that the specific growth rate in the Exponential plateau model is closer to the specific growth rate (μ) obtained from the experimental data (Table 2 and Fig 2). The data in Table 2 supports these findings. An accurate growth rate prediction model was developed using data from this study.

**Table 2.** Growth Performance of Microalgae Species

| | Maximum (Cells $mL^{-1}$) | Maximum absorbance | Maximum DW (g $L^{-1}$) | dt (d) | μ ($d^{-1}$) experimental | μ ($d^{-1}$) Gompertz | μ ($d^{-1}$) Logistic | μ ($d^{-1}$) Exponential plateau | μ ($d^{-1}$) Beta Growth |
|---|---|---|---|---|---|---|---|---|---|
| *Chlorella sorokiniana* | $2.64±3.02×10^7$ | 1.54±0.13 | 1.18±0.02 | 4.33±0.01 | 0.16 | 0.099 | 0.08 | 0.18 | 0.096 |
| *Chlorella* sp. | $2.63±3.08×10^7$ | 1.72±0.13 | 1.24±0.22 | 4.95±0.03 | 0.14 | 0.089 | 0.079 | 0.14 | 0.096 |

### 3.3. Predictive models

(Fig 3, 4, and 5) show the correlation between the optical-cell density, optical density-dry weight, and cell density-the dry weight of the tested microalgae species in this study. In these figures, the calculated predictive values are represented by lines.

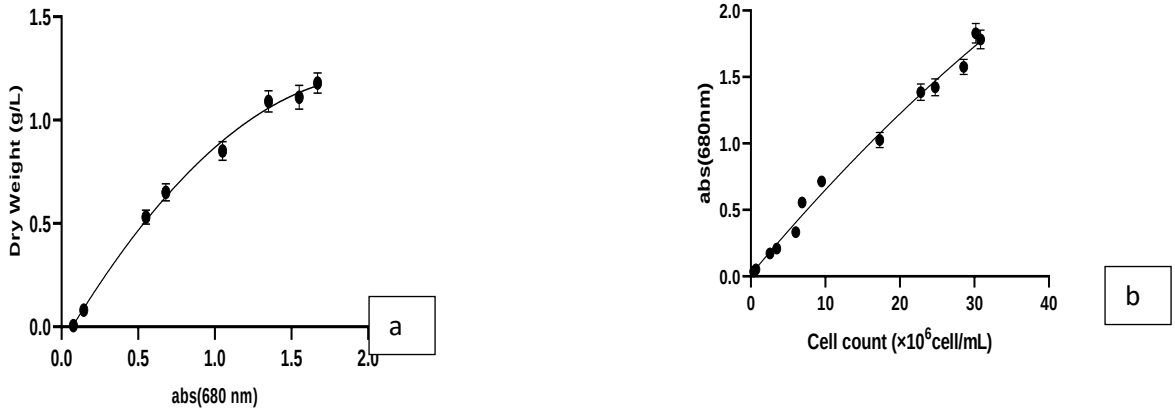


**Figure 3**. Relationship between absorbance at 680 nm and cell counting (cells $mL^{-1}$) for a. Chlorella sorokiniana and b.Chlorella sp. Black dots represent the experimentally observed values, and lines represent the theoretical values calculated using the developed predictive models.

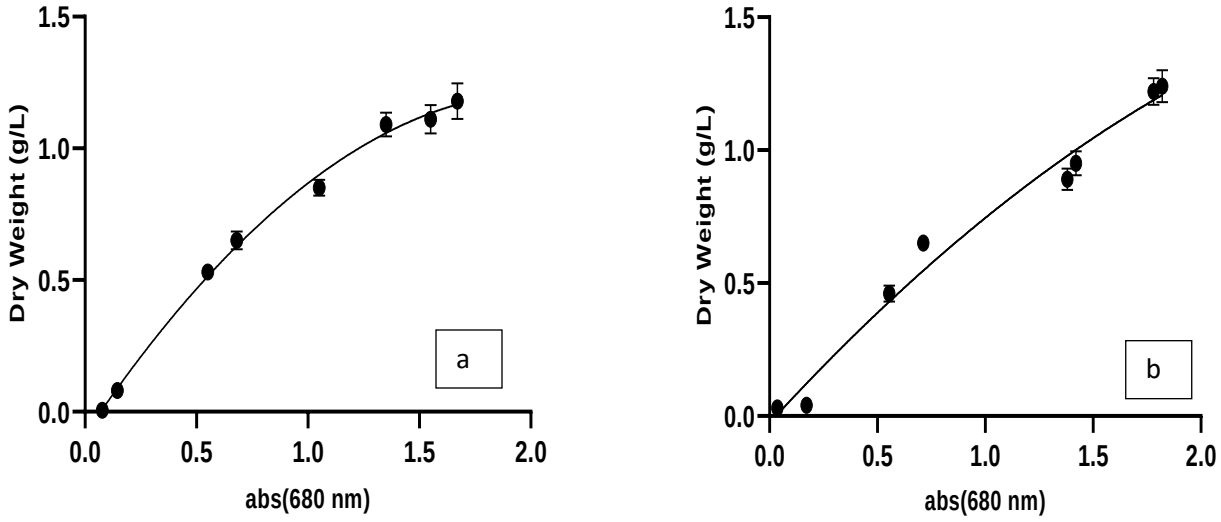


**Figure 4.** Relationship between absorbance at 680 nm and dry weight (g $L^{-1}$) for a. *Chlorella sorokiniana* and b. *Chlorella* sp. Black dots represent the experimentally observed values and lines represent the theoretical values calculated using the developed predictive models.

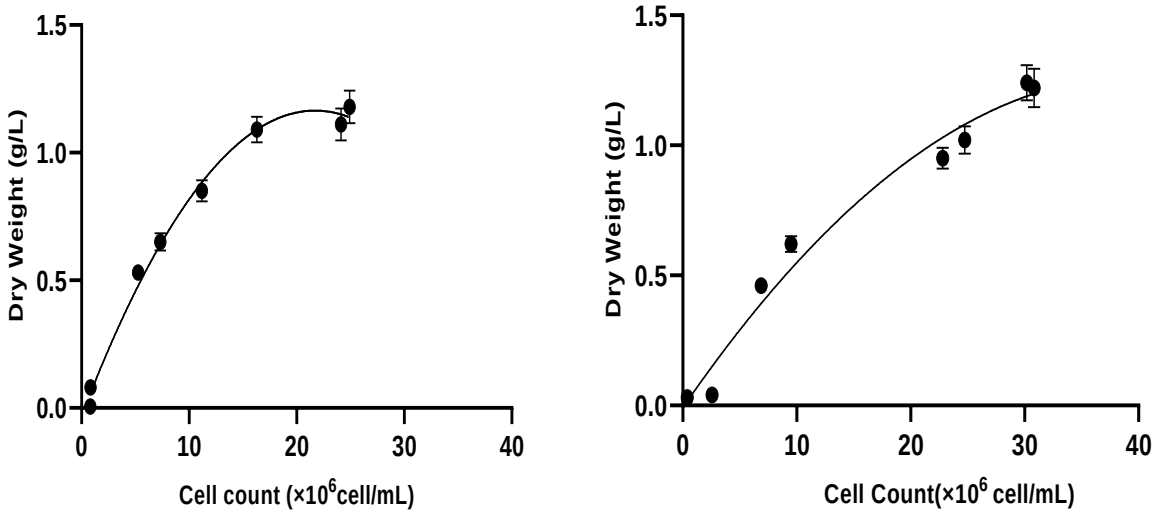


**Figure 5.** Relationship between counting (cells $mL^{-1}$) and dry weight (g $L^{-1}$) for a. *Chlorella sorokiniana* and b.*Chlorella* sp. Black dots represent the experimentally observed values and lines represent the theoretical values calculated using the developed predictive models.

Cell counts (cells $mL^{-1}$) and dry weight (g $L^{-1}$) were determined as functions of absorbance using predictive models developed by solving the previous equations at the appropriate absorbance values at 680nm for each microalgae species:

$$Cells\ mL^{-1} = \{[B_1^2 - 4B_2(B_0 - Abs\ 680)]1/2] - B_1\}/2B_2$$

(16)

$$Dry\ Weight(g\ L^{-1}) = \{[B_1^2 - 4B_2(B_0 - Abs\ 680)]1/2] - B_1\}/2B_2$$

(17)

Also, the predictive models, at the correspondent cell count (cells $mL^{-1}$) for each microalgae species, calculated dry weight (g $L^{-1}$) as a function of cell counting as follows:

$$Dry\ Weight(g \cdot L^{-1}) = \{[B_1^2 - 4B_2(B_0 - Cells.mL^{-1})]1/2] - B_1\}/2B_2$$

(18)

The regression coefficients of the absorbance and cell number (cells $mL^{-1}$) forecasting models are shown in Table 3. With a large coefficient of variance (0.991 and 0.986 for *C. sorokiniana* and *Chlorella* sp.), the absorbance of the microalgae suspensions explains more than 90% of the correlation with the cell density (cells $mL^{-1}$).

The validation of two microalgae strains' biomass production models demonstrates a high level of statistical accuracy with an RMSE of approximately 6% for both strains and a regression coefficient between the actual and predicted values (slope) of 0.988 and 0.991 for *C. sorokiniana* and *Chlorella* sp., respectively (Table 3). In addition, as shown in Table 3, the regression coefficients for *C. sorokiniana* and *Chlorella* sp. varied from 0.9966 to 0.9804, indicating that measured dry weight (g $L^{-1}$) has a significant correlation with cell density (cells $mL^{-1}$). The RMSE of 2% confirmed the high accuracy of the predictive model.

Also, by estimating dry weight based on cell concentration, the predictive model was validated and shown to be highly accurate (RMSE 3-7%); it also explained more than 95% of the variance (regression coefficients 0.9929 and 0.9726 for *C. sorokiniana* and *Chlorella* sp., respectively).

**Table 3.** Regression coefficients of the predictive models for A. abs and cell counting (cells $mL^{-1}$), B. abs and dry weight (g $mL^{-1}$), and C. cell counting (cells $mL^{-1}$) and dry weight (g $mL^{-1}$).

β0, β1, and β2 are regression coefficients for Eqs. (3) and (5). RMSE is the root of the mean square error. $R^2$ adj is the adjusted determination coefficient. Regression coefficients of the predictive models for A. abs and cell counting (cells $mL^{-1}$), B. abs and dry weight (g $mL^{-1}$), and C. cell counting (cells $mL^{-1}$) and dry weight (g $mL^{-1}$).

| Regression coefficients | *Chlorella sorokiniana* | | | *Chlorella sp.* | | |
|---|---|---|---|---|---|---|
| | A | B | C | A | B | C |
| $\beta_0$ | 0.009 | -0.086 | -0.031 | 0.0137 | -0.025 | -0.008 |
| $\beta_1$ | 0.085 | 1.25 | 0.11 | 0.066 | 0.885 | 0.063 |
| $\beta_2$ | -0.0008 | -0.30 | -0.002 | -0.0003 | -0.114 | -0.0007 |
| RMSE | 0.064 | 0.026 | 0.038 | 0.061 | 0.066 | 0.074 |
| $R^2$ | 0.9886 | 0.9966 | 0.9929 | 0.9918 | 0.9804 | 0.9766 |
| $R^2$ adj | 0.9860 | 0.9952 | 0.9901 | 0.9901 | 0.9726 | 0.9673 |
| AICc | -53.05 | -37.69 | -31.83 | -60.50 | -22.99 | -21.23 |

As illustrated in (Fig 6), the percentile deviation ([observed-expected]/observed.100) was less than 5(%) for *C. sorokiniana*, ranging from $3.46\times10^6$ to $2.49\times 10^7$ cells $mL^{-1}$, and roughly $0.39\times10^6$ to $2.47\times10^7$ cells $mL^{-1}$ for *Chlorella* sp. The predicted values for *C. sorokiniana* showed greater deviations from the experimental values at low cell density. Therefore, estimation of cell counting (cells $mL^{-1}$) by absorbance at a specific wavelength can be accurately performed within these cell density ranges for each microalgal species. According to equation (16), it can be seen that absorption in high concentrations is proportional to cell concentration. Due to the size

difference of microalgae cells, it is reasonable to assume that at high cell concentrations, the absorbing cells prevent some subsequent cells from interacting along the pathway (Thatipamala & Hill, 1991). As a result, estimates of cell concentration will be inaccurate due to light absorption at high concentrations. These findings corroborate the findings of a study conducted by Su et al. (2016), who used a 660 nm wavelength to analyze the cellular proliferation of two microalgal species (*C. vulgaris* and *Phormidium* sp.). Furthermore, Jia et al. developed a sensor for precise microalgae biomass concentration monitoring using OD measurements at 650, 685, and 780 nm; the growth rates calculated at each wavelength were deemed to be good indicators for tracking microalgae growth transitions and picking up on disturbances in the culture system (2015). In agreement with the estimation of cell density from absorbance data, Rodrigues et al. used a power function to produce an equation using absorbance data for precisely estimating *Pseudokirchneriella subcapitataof* up to $5\times10^6$ cells $mL^{-1}$ (Rodrigues et al., 2011). Also, Santos-Ballardo et al. established a correlation between spectrophotometric absorbance and cell counting (cells $mL^{-1}$) of *Isochrysis affinis galbana (T-Iso), Chaetoceros calcitrans, Nannochloropsis gaditana,* and *Phaeodactylum tricornutum* and presented equations with a confidence interval of 95% for microalgae densities of up to $10^7$(cells $mL^{-1}$) (2015). In this regard, Gomez et al. devised a straightforward method for measuring cell density using spectrophotometric absorbance in both batch culture and batch feeding of *Isochrysis galbana* during the exponential growth phase (2015).

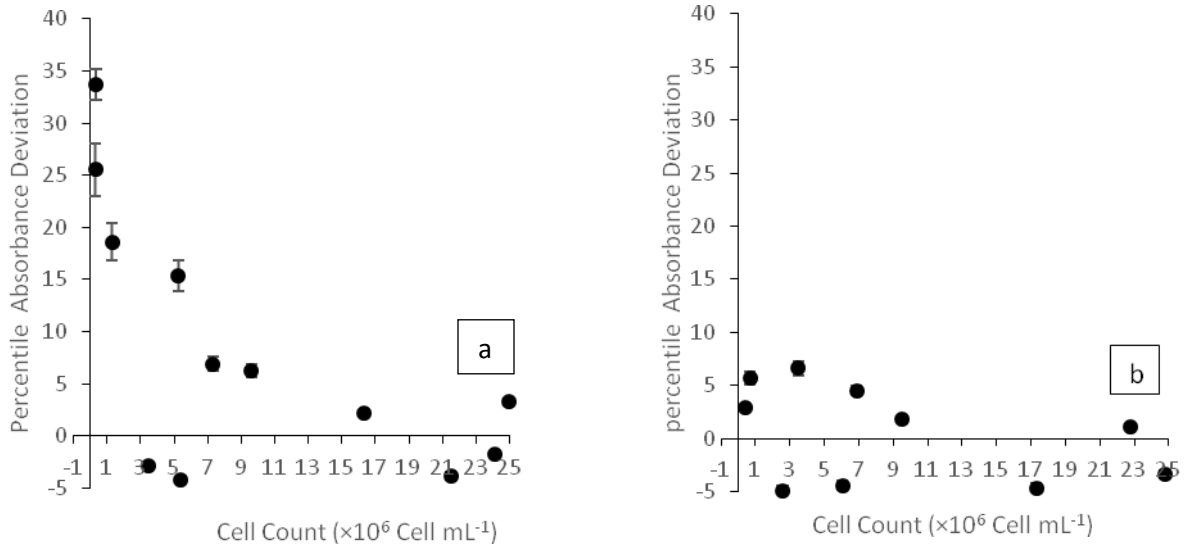


**Figure 6.** Percentile deviation [(observed−predicted) × 100 / observed] of the proposed model for the absorbance at 680 nm as of cell density (cells $mL^{-1}$) of a. *Chlorella sorokiniana* and b. *Chlorella* sp.

The percentile deviation (PD) of the fitted model for dry weight and absorption at 680 nm for each microalga is shown in (Fig 7). For dry weights up to 1.18 and 1.25 g $L^{-1}$ with a random distribution pattern for *Chlorella sorokiniana* and *Chlorella* sp., the percentile deviation is less than 10%. The adjusted model for *Chlorella sorokiniana* is significantly biased if it is less than 0.02 g $L^{-1}$. To calculate the dry weight of microalgae using optical density, the optical characteristics of cells must be considered because they are affected by their pigment content due to growth conditions and culture age, altering the relationship between absorbance and dry weight (Griffiths et al., 2011). Another source of inaccuracy could be the risk of bacterial contamination while determining the dry

weight of microalgae. Griffiths et al. (2011) found that using a standard curve constructed at a single time point in the development cycle to compute dry weight from optical density resulted in an average relative error across the growth cycle, relative to actual dry weight, ranging from 9 to 18 percent at 680 nm, which is consistent with the finding of this study. Also, Cordova et al. correlated the dry weight concentration of *C. sorokiniana* to the optical density at 600 nm (2018). Ajala and Alexander found a link between dry weight and correlation coefficients ($R^2$) of 0.986, 0.999, and 0.959 for *S. obliquus*, *C. vulgaris*, and *O. minuta*, respectively, with the optical density, OD580 (2020). According to Hotos et al., the most reliable wavelengths for estimating dry weight in *Nephroselmis*, *Amphidinium*, and *Phormidium* were 680 nm and 570 nm, respectively (2020).

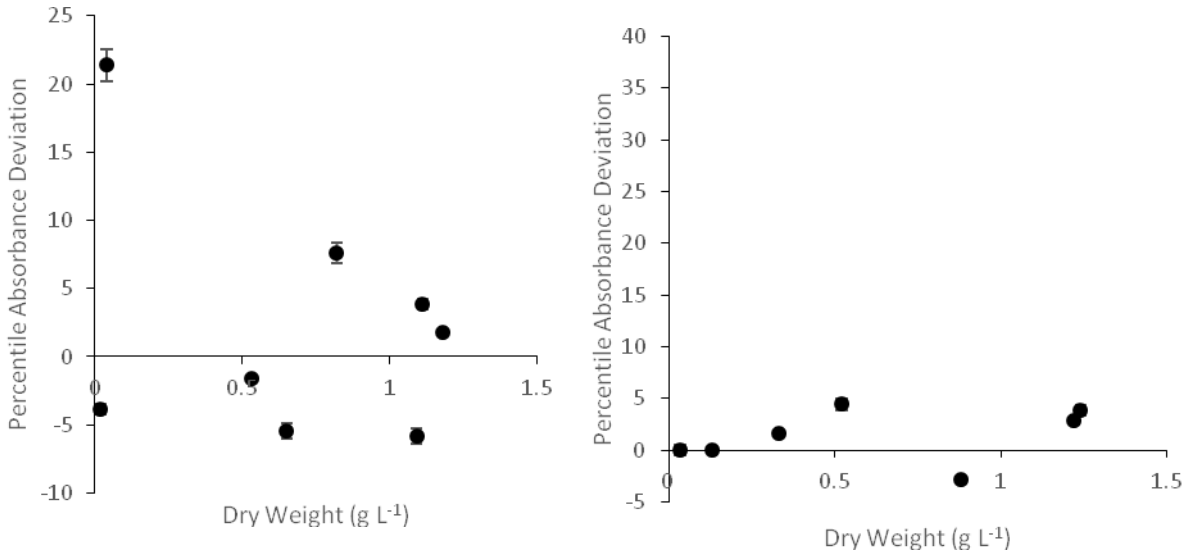


**Figure 7.** Percentile deviation [(observed−predicted) × 100 / observed] of the proposed model for the absorbance at 680 nm as of dry weight (g $L^{-1}$) of a. Chlorella sorokiniana and b. Chlorella sp.

To improve the accuracy and utility of the prediction equations provided in this study, the percentile deviation of cell density (cells $mL^{-1}$) and dry weight (g $L^{-1}$) of two microalgae strains with the least standard error and deviation were investigated. The percentile deviation between the predicted and observed cell density and dry weight is depicted in (Fig 8). As can be seen, the percentile deviation decreased by 7% between 0.006 and 1.18 g $L^{-1}$ for *C. sorokiniana*, and by 10% between 0.13 and 1.24 g $L^{-1}$ for *Chlorella* sp. Therefore, within these cell density ranges, it is possible to accurately estimate dry weight for the studied microalgae strains. Cell density changes as the cell water content changes. The ratio of biopolymers, such as carbohydrates, to the small monomers that make them up, determines the cytoplasmic osmolality. Biopolymer cleavage raises cellular ion content and hydration. Cellular hydration affects biomass dry weight more than wet density because hydrated biopolymers have a density similar to water (Chioccioli et al., 2014). The amount of cellular mucilage explains the inaccuracy when dry weight is determined using cell numbers. The amount of cellular mucilage present at different stages of growth affects the dry weight value of microalgae (Mahlmann et al., 2008). As a result, this inaccuracy may impact the estimation of cell dry weight by cell number.

The created models performed very well overall, covering a wide range of microalgae kinetics with only a small percentile deviation. This means that absorbance data measured at the appropriate wavelength can be used to accurately calculate the cell count (cells $mL^{-1}$) and dry weight of *Chlorella sorokiniana* and *Chlorella* sp**.**

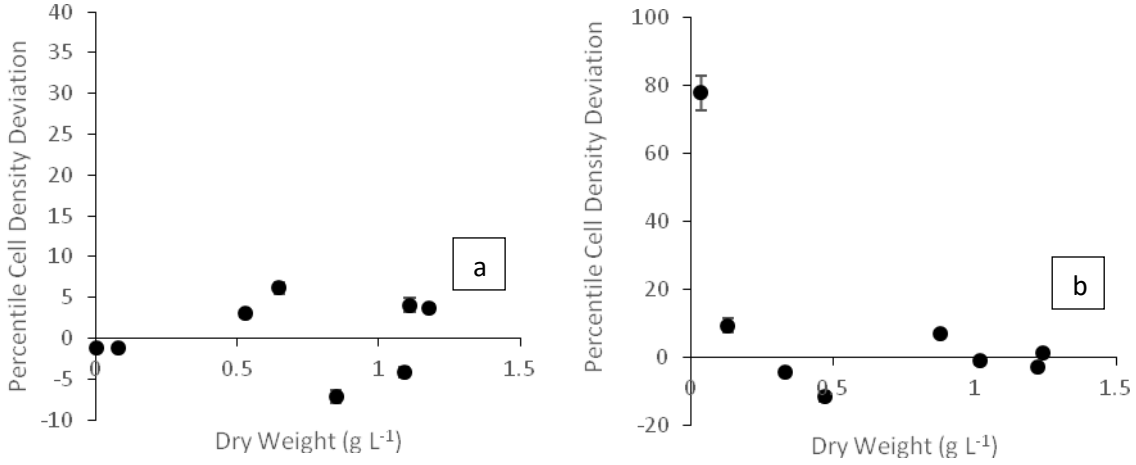


**Figure 8.** Percentile deviation [(observed−predicted) × 100 / observed] of the proposed model for the cell density as of dry weight (g $L^{-1}$) of a. Chlorella sorokiniana and b. Chlorella sp.

## 4. Conclusion:

Two marine microalgae species isolated from the Persian Gulf and Qeshm Island *C. sorokiniana* and *Chlorella* sp. were cultivated in BBM to determine optical density at multiple wavelengths (600, 640, 680, and 750 nm). The optimal wavelength for maximal absorbance was established for both microalgae strains. The growth modelling of *C. sorokiniana* and *Chlorella* sp. was investigated using four mathematical growth models (Logistic, Gompertz, Exponential plateau, and Beta growth). A high degree of agreement was found between the mathematic growth model and the experimental OD measurements. The spectrophotometric method, a simple and useful tool, was developed for the estimation of cell counting and dry weight. However, cell density estimation using the spectrophotometric method was found to be specific to each strain of algae and cannot be attributed to all types of algae, with regard to the morphology of the species and cell volume values. To this end, we confirmed the feasibility of estimating cell density by cell number and dry weight using the spectrophotometric method by calibrating a predictable and satisfactory model for a wide range of cell densities for *Chlorella* sp.and *C. sorokiniana*.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Ethical approval

This article contains no studies with human participants or animals performed by any of the authors.

## Open access



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