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MATHEMATICAL MODELING AND SIMULATION OF MIXOTROPHIC TETRADESMUS OBLIQUUS GROWTH

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Abstract. *The rising concern over greenhouse gas emissions calls for sustainable solutions to address the problem. One promising approach is the use of microalgae to treat emissions, as they efficiently absorb carbon dioxide and produce valuable biomass for renewable energy. However, scaling up microalgae production from the lab to commercial level poses challenges. Achieving high biomass productivity is difficult due to the sensitivity of microalgae growth to environmental factors like light, temperature, and nutrients. To address this, mixotrophic growth, using organic and inorganic carbon sources simultaneously, has shown promise in increasing productivity. This paper aims to develop a mathematical model to predict the growth of Tetradesmus obliquus in a mixotrophic culture. It will use glucose and atmospheric air as carbon sources in a controlled environment to validate the model. The study seeks to uncover optimal conditions for high biomass productivity through mathematical modeling and simulation, enhancing our understanding of microalgae growth mechanisms and optimizing mixotrophic cultures.*

Keywords: *microalgae, computational simulation, mixotrophic, glucose.*

1. INTRODUCTION

Microalgae are unicellular photosynthetic aquatic microorganisms, through their photosynthetic metabolism, these microorganisms effectively assimilate carbon dioxide and light to generate oxygen and obtain vital carbon sources and energy for metabolic processes. The abundance of lipids and carbohydrates within microalgae biomass give them considerable promise for sustainable biofuel production, like biodiesel, ethanol and most recently kerosene (Costa *et al.*, 2022). Moreover, owing to their capability in effectively absorbing and utilizing carbon dioxide from emissions, microalgae present a promising potential for mitigating greenhouse gas pollution and treating industrial emissions. Beyond their bioenergy applications, microalgae's rich protein content renders them a viable resource for animal feed and nutraceutical supplementation. Additionally, these microorganisms serve as a prolific source of pigments, carotenoids, and other valuable metabolites, eliciting considerable scientific interest and exploration in various interdisciplinary sectors (Siaut *et al.*, 2011).

Autotrophic and heterotrophic growth are two distinct strategies employed by microalgae to sustain their development and metabolism. Autotrophic microalgae utilize photosynthesis as their primary means of sustenance, converting carbon dioxide and light into organic compounds and oxygen. Conversely, heterotrophic microalgae rely on organic carbon sources, such as sugars and other compounds, as their main energy and carbon supply. Unlike autotrophs, they do not require light and can grow in darkness or low-light conditions. This makes heterotrophic growth particularly advantageous in controlled bioreactor systems, where light limitation or fluctuating environmental conditions may impede autotrophic growth. While autotrophic microalgae have the benefit of utilizing sunlight and atmospheric CO2, heterotrophic microalgae can achieve higher growth rates and biomass production, but at the cost of an external carbon source dependency (Zhan, 2017)

Unlike solely autotrophic or heterotrophic approaches, mixotrophic growth allows microalgae to simultaneously utilize both organic carbon sources and inorganic carbon. This flexibility enhances their metabolic efficiency and enables them to thrive in diverse environmental conditions. Mixotrophic cultures often exhibit higher growth rates and increased biomass productivity compared to pure autotrophic or heterotrophic cultures. Additionally, mixotrophic microalgae can effectively remove excess nutrients, such as nitrogen and phosphorus, from wastewater or other effluents. (Lourenço, 2006; Zhan, 2017).

Achieving an economically viable production of microalgae necessitates the exploration of competitive methods that can be seamlessly integrated with existing energy infrastructures, such as thermoelectric plants. The upscaling of microalgae cultivation from the laboratory to industrial level is a big challenge, the production of high concentration microalgae cultures using mixotrophic metabolism holds great promise as an innovative solution to address this hurdle. Mathematical modeling and simulation play a pivotal role in optimizing this process, providing crucial insights to enhance productivity and establish a foundation for large-scale industrial applications.

The objective of this work is to model and simulate mixotrophic *Tetradesmus obliquus* growth using glucose as organic carbon source and atmospheric air as inorganic carbon source, laboratorial data will be collected to validate the model.

2. MATHEMATICAL MODEL

The microalgae production and data collection for the validation of the mathematical model will be carried out at the NPDEAS (Sustainable Energy Research and Development Center) located in the Federal University of Paraná in Curitiba.

The mathematical model will be developed with the equations of the Mass Balance and Growth Kinetics. The validation experimental data will be made using microalgae produced in a laboratorial scale.

2.1 Mass Balance

The mass balance was conducted considering that both autotrophic and heterotrophic metabolism was happening at the same time, therefore two different biological reactions will be considered as shown in Figure 1.

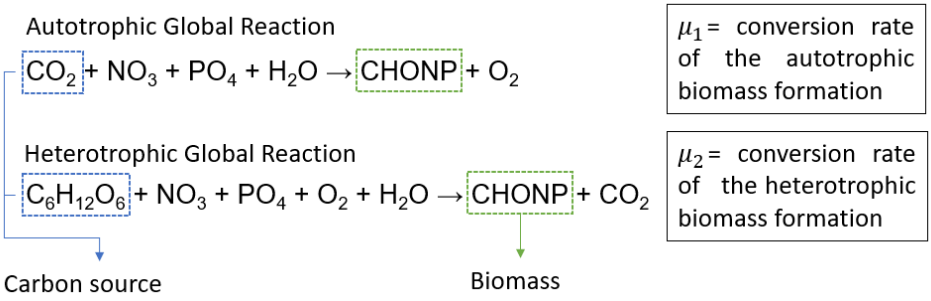


Figure 1. Simplified biomass formation reactions.

The biomass mass balance equation is described in the Eq. (1).

$$\frac{dX_i}{dt} = \frac{Q}{V}.X_0 + \mu_1.X_i + \mu_2.X_i, \quad (1)$$

Where X_i is the biomass concentration and μ_1 and μ_2 are the conversion rate of the biomass formation reactions.

The nitrate mass balance equation is described in Eq. (2).

$$\frac{d[NO_3]_i}{dt} = \frac{Q}{V}.[NO_3]_0 - Y_{\frac{NO_3}{X}}.\mu_1.X_i - Z_{\frac{NO_3}{X}}.\mu_2.X_i, \quad (2)$$

In the given expression, $[NO_3]$ represents the concentration of nitrate, while $Y_{\frac{NO_3}{X}}$ and $Z_{\frac{NO_3}{X}}$ denote the stoichiometric ratios of biomass formation reactions between nitrate and biomass.

The phosphate mass balance equation is described in Eq. (3).

$$\frac{d[PO_4]_i}{dt} = \frac{Q}{V}.[PO_4]_0 - Y_{\frac{PO_4}{X}}.\mu_1.X_i - Z_{\frac{PO_4}{X}}.\mu_2.X_i, \quad (3)$$

In the given expression, $[PO_4]$ represents the concentration of nitrate, while $Y_{\frac{PO_4}{X}}$ and $Z_{\frac{PO_4}{X}}$ denote the stoichiometric ratios of biomass formation reactions between phosphate and biomass.

The glucose mass balance equation is described in Eq. (4).

$$\frac{d[Glu]_i}{dt} = \frac{Q}{V}.[Glu]_0 - Y_{\frac{Glu}{X}}.\mu_1.X_i - Z_{\frac{Glu}{X}}.\mu_2.X_i, \quad (4)$$

In the given expression, [Glu] represents the concentration of nitrate, while $Y_{\frac{Glu}{X}}$ and $Z_{\frac{Glu}{X}}$ denote the stoichiometric ratios of biomass formation reactions between glucose and biomass.

The carbon dioxide dissolved in the liquid phase mass balance equation is described in Eq. (5).

$$\frac{d[CO_2]_i}{dt} = \frac{Q}{V}.[CO_2]_0 - Y_{\frac{CO_2}{X}}.\mu_1.X_i + Z_{\frac{CO_2}{X}}.\mu_2.X_i + h_{m_{CO_2}}.([CO_2]^* - [CO_2]_i), \quad (5)$$

Where $[CO_2]$ denotes the concentration of carbon dioxide dissolved in the liquid phase, while $Y_{\frac{CO_2}{X}}$ and $Z_{\frac{CO_2}{X}}$ signify the stoichiometric ratios of the biomass formation reactions between carbon dioxide and biomass. Additionally, h_{mCO2} represents the mass transfer coefficient, and $[CO_2]^*$ refers to the concentration of carbon dioxide at the interface, which can be calculated using Henry's Law as described in Eq. (6).

$$[CO_2]^* = H_{CO_2}.p_{CO_2}, \quad (6)$$

Where H_{CO2} is Henry's constant for CO_2 and p_{CO2} denotes the partial pressure of carbon dioxide in the gas entering the reactor.

The dissolved oxygen in the liquid phase mass balance equation is described in Eq. (7).

$$\frac{d[O_2]_i}{dt} = \frac{Q}{V}.[O_2]_0 + Y_{\frac{O_2}{X}}.\mu_1.X_i - Z_{\frac{O_2}{X}}.\mu_2.X_i + h_{m_{O_2}}.([O_2]^* - [O_2]_i), \quad (7)$$

Where $[O_2]$ is the concentration of dissolved oxygen in the liquid phase, $Y_{\frac{O_2}{X}}$ and $Z_{\frac{O_2}{X}}$ is the stoichiometric ratio of the biomass formation reactions between oxygen and biomass respectively, h_{mO2} is the mass transfer coefficient and $[O_2]^*$ is the oxygen concentration at the interface, which can be determined analogously to the carbon dioxide concentration at the interface.

2.2 Growth Kinetics

The growth kinetics of each reaction will be listed bellow in Eq. (8) and (9).

$$\mu_1 = \mu_{max}.\mu(T).\mu(I_0).\mu(CO_2).\mu(O_2).\mu(N_{Tot}).\mu(P_{Tot}), \quad (8)$$

$$\mu_2 = \mu_{max}.\mu(T).\mu(I_0).\mu(CO_2).\mu(O_2).\mu(N_{Tot}).\mu(P_{Tot}).\mu(Glu), \quad (9)$$

The autotrophic reaction considers the influence of temperature, light intensity, dissolved carbon dioxide, dissolved oxygen, total nitrate and total phosphate.

The heterotrophic reaction considers the same influences with the addition of the glucose to the equation.

Each term in the equations signifies the impact of these variables on the reaction rate. The specific equations to calculate each component of Eq. (8) and (9) can be found in Table 1.

Table 1. Growth kinetics equation variables.

Variable	Equation	Reference
Temperature	$\mu(T) = a.T^2 + b.T + c$	Balmant *et al*., 2011.
Light intensity	$\mu(I_O) = \frac{I_O}{\left(K_{I_O} + I_O + \frac{I_O^2}{ki_{I_O}}\right)}$	Aiba, 1982.
Carbon dioxide	$\mu(CO_2) = \frac{CO_2}{\left(K_{CO_2} + CO_2 + \frac{CO_2^2}{ki_{CO_2}}\right)}$	Andrews, 1986.
Oxygen	$\frac{A}{\left(1 + \left(\frac{O_2\%}{B}\right)^C\right)}$	Rampi *et al.*, 2023
Total nitrate	$\mu(N_{Tot}) = \frac{N_{Tot}}{\left(K_{N_{Tot}} + N_{Tot}\right)}$	Araújo *et al*., 2009.
Total phosphate	$\mu(P_{Tot}) = \frac{P_{Tot}}{\left(K_{P_{Tot}} + P_{Tot}\right)}$	Araújo *et al*., 2009.
Glucose	$\mu(Glu) = \frac{Glu}{(K_{Glu} + Glu)}$	This work.

All simulations will be performed using MATLAB® software.

2.3 Validation Experimental Data

The experimental data that will be used to validate the mathematical model will be made in a laboratory scale, using a controlled room with constant temperature, artificial light source trough white LED lamps turned on during all the experiments.

All the microalgae will be cultivated in synthetic medium Chu, the composition is listed in Table 2.

Table 2. Chu medium composition.

Component	Chemical Form	final Concentration ($g.L^{-1}$)
Sodium Nitrate	$NaNO_3$	0,25
Calcium Chloride Dihydrate	$CaCl_2.2H_2O$	0,025
Magnesium Sulfate Heptahydrate	$MgSO_4.7H_2O$	0,075
Dibasic Potassium Phosphate	KH_2PO_4	0,075
Monobasic Potassium Phosphate	K_2HPO_4	0,175
Sodium Chloride	NaCl	0,025
EDTA (Ethylene Diamine Tetraacetic Acid)	$C_{10}H_{16}N_2O_8$	0,05
Sodium Hydroxide	KOH	0,031
Ferrous Sulfate Heptahydrate	$FeSO_4.7H_2O$	0,005
Boric Acid	H_3BO_3	0,01142
Zinc Sulfate Heptahydrate	$ZnSO_4.7H_2O$	$8{,}82\times10^{-5}$
Manganese Chloride Tetrahydrate	$MnCl_2.4H_2O$	$1{,}44\times10^{-5}$
Sodium Molybdate Dihydrate	$NaMoO_4.2H_2O$	$7{,}1\times10^{-6}$
Copper Sulfate Pentahydrate	$CuSO_4.5H_2O$	$1{,}57\times10^{-5}$
Cobalt Nitrate Hexahydrate	$Co(NO_3)_2.6H_2O$	$4{,}9\times10^{-6}$

In addition to the Chu medium, it will also be added 10 $g.L^{-1}$ of anhydrous glucose.

The microalgae growth will be held in a 2L Erlenmeyer with 1.6L of culture, with constant air feeding to provide inorganic carbon source and an agitation method to avoid decantation. All the experiment will be made in triplicate. Figure 2 shows the same cultivation method that will be used in the experimental data collection stage.

Figure 2. Microalgae growth in Erlenmeyer.

Daily analysis will be held to follow the microalgae growth rate, microalgae morphology, glucose consumption rate, pH alterations and to check for possible contaminations.

The cell count will be performed using an optical microscope with a Neubauer chamber and a manual counter. The collected result represents the quantity of cells per mL of culture. Besides the quantitative value, observing the culture under a microscope also serves to track the stage of cell development and to analyze the possible presence of undesirable microorganisms that may appear.

Using a digital benchtop pHmeter, the pH of the culture will be measured. It is essential for this analysis to be conducted immediately after the collection because the sample undergoes metabolic changes that can alter the pH within a few minutes after collection. The pH meter used in the experiments can be observed in Figure 3.

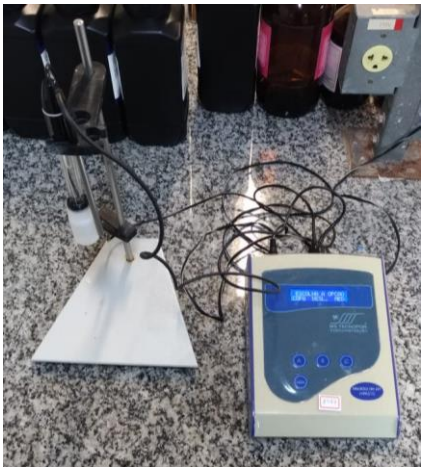


Figure 3. Digital pHmeter.

A spectrophotometer will be used to measure the sample's ability to absorb light, with readings taken at a wavelength of 420 nm, indirectly capturing the amount of chlorophyll present in the medium. The Lambert-Beer law demonstrates that the higher the sample's absorbance, the higher its concentration, in this case, cellular concentration. This measurement can be compared with the cell count.

The same spectrophotometer will be used to read the concentration of the glucose in the medium, using a enzymatic reagent, readings taken at a wavelength of 505 nm. Providing a way to track down how much glucose was consumed until the sample collection. The spectrophotometer used in the experiments can be observed in Figure 4.

Figure 3. Spectrophotometer.

The dry biomass quantifies the sample's concentration in grams of biomass per volume of culture. To determine this, fiberglass membranes are used, which have been previously dried in an oven at 60°C for 24 hours, and their mass is measured using a precision balance. Next, 10 mL of the sample is filtered using a Buchner funnel with a vacuum pump. The membrane with the retained biomass is left in the oven at 60°C for an additional 24 hours to remove all moisture. Afterward, the mass of the membrane is measured again using a precision balance. The difference in mass of the filter before and after filtration determines the amount of biomass in grams per 10 mL of culture.

3. RESULTS

The validation of the mathematical model was performed using experimental data. The results are presented as the mean of triplicate results from the proposed experiment. The graph illustrating the growth of microalgae biomass using Chu medium with 10g.L^{-1} of glucose according to the proposed model is shown in Figure 4.

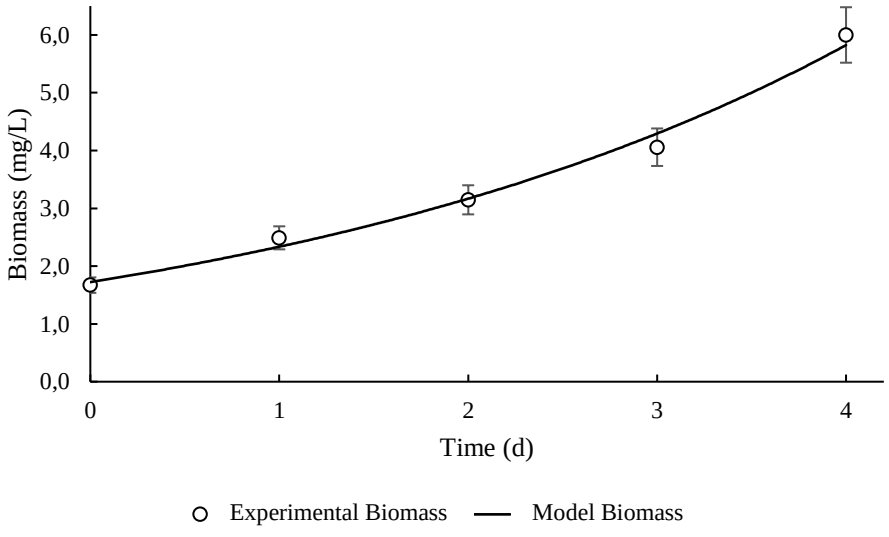
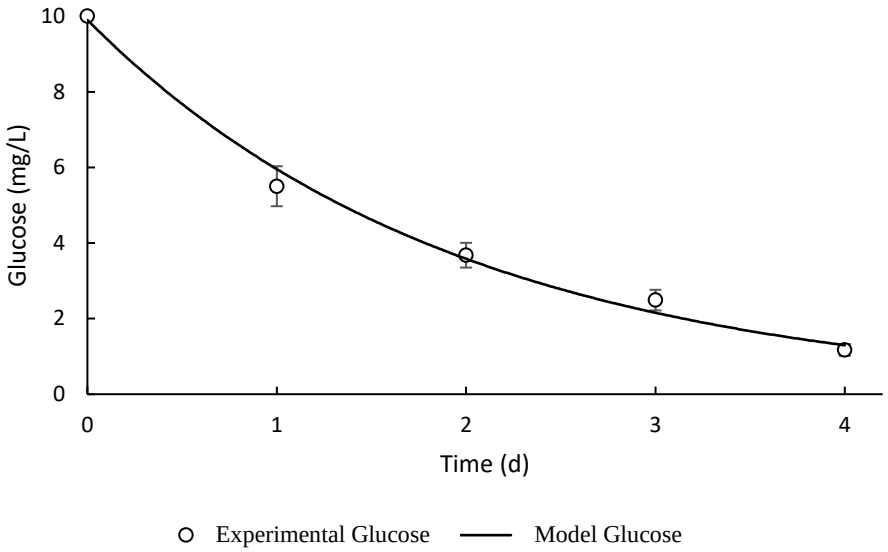


Figure 4. Experimental and theorical model biomass growth.

In the same simulation, the graphic representing the glucose consumption with the proposed model is presented in figure 5.



The graphics illustrates a concurrence between the model's predictions and the observed experimental trends, substantiating the model's ability to simulate microalgae biomass dynamics under specified glucose concentrations.

The simulation reveals a compelling alignment between the model and experimental findings, showcasing a robust approximation for both microalgae biomass growth and glucose consumption. This congruence between simulation and experiment underlines the model's efficacy in simulating and understanding the dynamics of microalgae growth and glucose utilization, emphasizing its potential for furthering research and applications in this field.

4. CONCLUSIONS

In summary, the constructed model exhibits robustness in simulating the diverse growth modes—autotrophic, heterotrophic, and mixotrophic—of microalgae, showcasing a close correlation with the gathered experimental data. This alignment affirms the model's competence in replicating the intricate dynamics of microalgae cultivation under various nutritional conditions. Nonetheless, to further fortify its reliability and comprehensiveness, it is essential to subject the model to additional validation tests. By adjusting glucose concentrations and applying the model to a broader spectrum of experimental scenarios, a more in-depth assessment of its parameters and behaviors across diverse settings can be achieved. This expanded validation process will not only refine the model but also facilitate a more comprehensive evaluation, enhancing its adaptability and precision in modeling microalgae growth under variable nutritional conditions.

This extended validation, which involves varying glucose concentrations and its application across different experimental studies, is crucial for refining the model's accuracy and generalizability. Through these tests, a deeper comprehension of how the model responds to alterations in nutrient availability can be gained, enabling a more thorough analysis of its performance and robustness. Such enhancements will ultimately contribute to a more versatile and reliable tool for predicting microalgae growth under varying nutrient conditions, offering insights into their behavior across a wide spectrum of experimental setups.

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