**Comparison of kinetic models for *Chlorella vulgaris* growth in mixotrophic culture**

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**1.Introduction**

In the past few years, microalgae have become a promising candidate for large-scale biodiesel production, which is a solution for carbon dioxide emissions and energy shortages [1]. Despite many advantages, microalgae biodiesel production has not yet been commercialized due to low lipid productivity and the high cost of feedstock compared to diesel produced from fossil fuels [2]. Furthermore, biodiesel production from microalgae is highly dependent on the availability of biomass and the scale-up process, which must be optimized to reduce costs. Kinetic models simplify designing a bioreactor, controlling microbial processes, and predicting their behavior more easily than laboratory experiments [3]. Few reports are available on kinetic modeling of algae growth and lipid production under mixotrophic conditions. The Luedeking-Piret equation can adequately explain microalgae growth kinetics. It is an unstructured model, and growth and non-growth related contributions are combined towards product formation [4]. In this study, growth and substrate consumption kinetics of *Chlorella vulg*aris were modeled according to the distinguished Ludeking-Piret mathematical model with four different kinetics of Monod, Logistic, Tessier, and Bolton. Different parameters of KS, KP, µmax, YX/S, YP/S, α, and β were estimated to obtain the most meaningful results and the most efficient model to describe growth, lipid production, and substrate consumption.

**2. Methods**

*C. vulgaris*was grown in a synthetic medium with 480 mg L-1 COD (chemical oxygen demand), 200 mg L-1 TN (total nitrogen), and 14 mg L-1 TP (total phosphorus) for 12 days. Samples of the culture were centrifuged, and supernatants were analyzed for COD, TN, and TP [5]. Total lipids were determined according to the modified Bligh and Dyer method [6].

The growth, product, and consumption of substrates (COD, TN, and TP) were predicted according to equations (1), (2), and (3). Equations (2) and (3) are known as Luedeking-Piret equations.

 $\frac{dX}{dt}=μX$ (1)

 $ \frac{dP}{dt}=∝\left(\frac{dX}{dt}\right)+βX$(2)

 $ -\frac{dS}{dt}=\frac{1}{Y\_{^{X}/\_{S}}}\left(\frac{dX}{dt}\right)+mX+\frac{1}{Y\_{^{P}/\_{S}}}\left(\frac{dP}{dt}\right)$(3)

where X is the biomass concentration (g L-1), t is the growth time (days), µ is the specific growth rate (day-1), P is the lipid concentration (g L-1), α is the lipid formation coefficient, and β (day-1) a non-growth correlation coefficient, S is the concentration of rate-limiting substrate (COD, TAN, and TP) (g L-1), Yx/s is the growth yield coefficient; Yp/s is the lipid yield coefficient, m is the maintenance coefficient (day-1). These parameters are correlated with biomass production rate (dX/dt), and therefore, solving them simultaneously results in more accurate results. The mentioned growth kinetic models are used to determine μ. The ordinary differential equations were solved by MATLAB software, and the predicted results were compared with experimental data.

 **3. Results and discussion**

The Monod equation for phosphorus, which has the lowest concentration relative to COD and nitrogen, has calculated the lowest μmax (0.87 d-1), which is not valid enough to determine the correlation coefficient R2 (0.72). This model has reached a suitable R2 for COD and nitrogen and has calculated a significant μmax (1.48 d-1). Due to the exponential term, the Tessier equation includes the effect of severe inhibition of the substrate. However, when the substrate concentration is low in the medium, the comprehensiveness of the model in determining the kinetic parameters is well estimated, and the logical values ​​for the kinetic parameters are calculated. The logistic equation in determining biomass growth is independent of the effect of substrate and product. Since the substrate concentrations were low and within the standard range, it is natural that no growth inhibition was observed in the medium. Compared to other models, this model best fits experimental data (R2 > 0.98) and predicts the highest YX/S (137.7 g/g)for phosphorus. The Bolton equation is independent of the concentration of the substance and includes the inhibitory parameter of the product. This model calculates the lowest μmax compared to other models. When the microalgae are in the growth phase, there is little change in the intracellular lipid content. As the biomass concentration increases, the amount of lipid in the medium also increases. Therefore, in reality, the product does not act as a deterrent to microalgae growth. In general, in the models that strongly (or even relatively) attributed the inhibition to the initial concentrations of the substance, there was no good agreement in determining the concentration of biomass or substrate with its practical reality.



 **Figure 1.** Fitting of biomass concentrations, lipid production and COD on logistic model

 (Blue diagrams are experimental data)

**4. Conclusions**

Examining the obtained results based on the performed modeling, it was found that the logistic model with a fitting coefficient of 0.98 has a good prediction. The greater complexity of the equations and the increase of the effective parameters will not increase the accuracy of their predictions but will sometimes deviate from the correct estimation.

**References**

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