

# Predictive Growth Model of Microalgae in Agricultural Wastewater

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## Abstract

Agricultural wastewater is a dark brown viscous liquid with a high concentration of degradable organic matter and mineral constituents. The incorporation of microalgae in agricultural wastewater to utilize organic substances creates a potentially unprecedented valuation. However, provided that the descriptive growth model of microalgae in agricultural wastewater is rarely addressed. In this study, an assessment was performed on four mathematical models (Logistic, Modified Logistic, Modified Gompertz, and Baranyi-Roberts) on the growth of *Coellastrela* sp. UKM4, *Chlamydomonas* sp. UKM6 and *Scenedesmus* sp. UKM9 based on the available published works in the literature. Statistical analyses, including  $R^2$ , adjusted  $R^2$ , root-mean-square error (RMSE), bias factor (BF), accuracy factor (AF), and percent of standard error prediction (%SEP) were applied to verify the accuracy of each model. The findings showed, based on the visual analysis and residual plots, modified logistic model and Baranyi-Roberts model had produced good fitting curve between experimental data and model predictive data. For statistical analysis, both models produced  $R^2$  (>95%), adjusted  $R^2$  (>93%), RMSE (0.048-0.08), BF (0.9-1.01), AF (<1.1) and %SEP (7.7%-15%). The findings therefore revealed that the most appropriate model for predicting the growth of *Coellastrela* sp. UKM4, *Chlamydomonas* sp. UKM6 and *Scenedesmus* sp. UKM9 in agricultural wastewater were modified logistic and Baranyi-Roberts models.

## 1. Background of study

Agriculture industries is an important sector of Malaysia's economy, contributing 12 percent to the national GDP and providing employment for 16 percent of the population (Foo et al., 2019). Indirectly, the rapid development of the agriculture industry also increased the release of untreated wastewater. As reported in previous research, agricultural wastewaters consist of high organic and inorganic compound such as COD, BOD, nitrogen, phosphorus and minerals, which can have adverse effects on human health and the environment (Foo et al., 2019).

Microalgae are a complex group of photosynthetic organisms whose systematics focus on the types and variations of photosynthetic pigments found in different species. Recently,

application of microalgae as a bioremediation agent has attracted the attention of researchers as they can grow under a desolate environment without affecting their productivity (Ansari et al., 2019). The richness of organic carbon, nitrogen and phosphate in agricultural wastewater has made it possible to boost the growth of microalgae in the producing of massive biomass for downstream applications (Ahmad et al., 2016). However, the selection of a suitable mathematical model for assessing the growth of microalgae in agricultural wastewater has not been reported. This is crucial for a better understanding of the behaviour and potential utilisation of microalgae in different environment.

According to Zwietering et al. (1990), the growth rate of microalgae in different environments can be determined by the adoption of bacterial growth modelling as proposed, which was based on a mathematic model using constants such as a, b, and c (Korkmaz, 2016). Then, the parameters were modified using maximum value (A), maximum growth rate/growth maximal value ( $\mu_{max}$ ), and lag time ( $\lambda$ ) to fit the biological significance (Kim & Jeong, 2017). To date, numerous growth models have been proposed to explain the bacterial growth rates, including the logistic, Schnute, Stannard, Richards, and Gompertz models (Çelekli, Balci, & Bozkurt, 2008). In the current study, four different mathematical models were selected, namely the logistic, modified logistic, modified Gompertz, and Baranyi-Roberts models, to evaluate the microalgae growth profile under controlled culture conditions (Kawano, Wallbridge, & Plummer, 2020) (Schmidt, Gagnon, & Jamieson, 2016).

Logistic and modified logistic models by Vogels et al. (1975) is a model which is independent of substrate intake and is represented by a sigmoid shaped curve (Chang et al., 2016). Besides, the modified Gompertz model is employed from the original Gompertz equation to incorporate the lag phase to produce an ideal mathematical model for different microbial growth curves (Division et al., 2016). The Baranyi-Roberts model was used to detect the maximum specific growth rates for microalgae. This model has four parameters in its formula compared to the modified logistical model and modified Gompertz model have only three parameters.

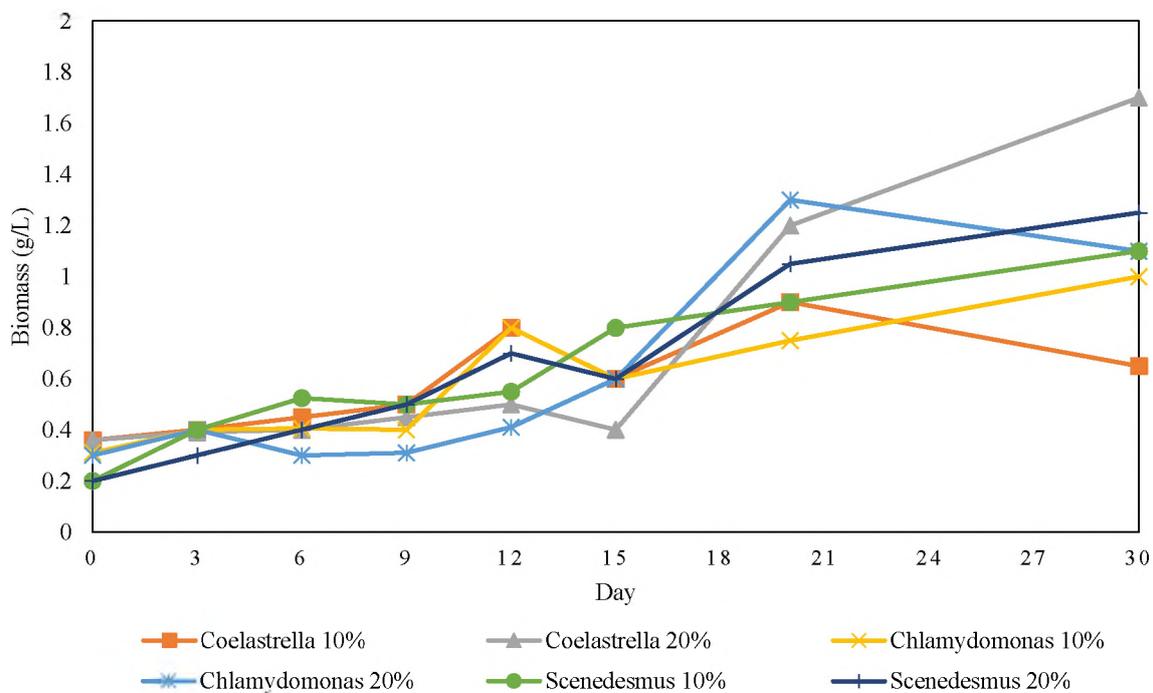
Therefore, the purpose of this analysis was to predict the best growth models for three local microalgae, *Coellastrella* sp. UKM4 (10% and 20%), *Chlamydomonas* sp. UKM6 (10% and 20%) and *Scenedesmus* sp. UKM9 (10% and 20%) in agricultural wastewater with four different growth models: logistic, modified logistic, modified Gompertz and Baranyi-Roberts

models. The credibility of each of the models was assessed on the basis of visual analysis, residual plot analysis and statistical performance.

## 2. Methodology

### 2.1 Acquisition of Data

In this study, data were obtained from previous work by Mohd Udaiyappan et.al (2020). The growth of three different microalgae, *Coellastrella* sp., *Chlamydomonas* sp. and *Scenedesmus* sp., in agricultural wastewater was measured on the basis of biomass produced over 30 days and then replotted as shown in Figure 1.



**Figure 1:** Growth profile of *Coellastrella* sp., *Chlamydomonas* sp. and *Scenedesmus* sp. Replotted from Mohd Udaiyappan et.al., (2020)

### 2.2 Fitting of the data

Growth data were fitted nonlinearly using MATLAB (Version R2020b) with a curve fitting command based on the Levenberg-Marquardt algorithm. This algorithm calculates the starting values by searching for the steepest ascent on the curve and uses four datum points to estimate the  $\mu_{max}$ . Thus, this algorithm is capable of minimising the sums of square of residuals between the predicted and experimental values and producing a good fitting curve.

## 2.3 Mathematical Models

Four mathematical models namely logistic, modified logistic, modified Gompertz, and Baranyi Roberts were employed to compare the best-fit growth model for *Coellastrella* sp., *Chlamydomonas* sp. and *Scenedesmus* sp. cultivated in agricultural waste.

### 2.3.1 Logistic Model

The logistic model explains microbial growth based on the original population density, time, growth rate, and density of the final population. Pearl and Reed (1920) developed the original logistic function model. The logistic model expresses itself as follows:

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

where A is the asymptotic of  $\ln X_t/X_0$  as t decreases indefinitely, C is the asymptotic of  $\ln X_t/X_0$  as t increases indefinitely, B is the relative growth rate at time M ( $\text{day}^{-1}$ ), t is the residence time (day), M is the time at which the maximum growth rate is reached (day),  $X_t$  is the biomass concentration at time t ( $\text{g L}^{-1}$ ) and  $X_0$  is the initial biomass concentration ( $\text{g L}^{-1}$ ). On this basis, the logistic expression become:

$$X(t) = \frac{X_0 e^{(\mu_{max} \cdot t)}}{[1 - \left(\frac{X_0}{X_{max}}\right) (1 - e^{(\mu_{max} \cdot t)})]} \quad (2)$$

### 2.3.2 Modified Logistic Model

This model is a mechanistic model that has been upgraded from a logistic empirical model. In quantitative parameters, microbiological concepts such as lag phase ( $\lambda$ ) and maximum growth rate ( $\mu_{max}$ ) have been integrated as expressed in the following equation:

$$X(t) = X_0 + \frac{(X_{max} - X_0)}{[1 - \exp\left\{\left(\frac{4\mu_{max}}{(X_{max} - X_0)}\right) (\lambda - t) + 2\right\}]} \quad (3)$$

Where,  $X_0$  and  $X_{max}$  are the initial and maximum biomass concentration (g/L) and  $\mu_{max}$  refers to the maximum growth rate ( $\text{day}^{-1}$ ), while  $\lambda$  refers to the lag phase (day).

### 2.3.3 Modified Gompertz Model

Modified Gompertz model was based on Gompertz equation. The equation was modified to use in defining microorganism inactivation (Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990). Through this model, a few biological parameters are used in equation:

$$X(t) = X_0 + (X_{max} - X_0) \exp \left\{ -\exp \left[ \frac{\mu_{max} \exp(1)}{(X_{max} - X_0)} (\lambda - t) + 1 \right] \right\} \quad (4)$$

From this equation,  $\lambda$  also call lambda is refer to lag phase (day), meanwhile  $\mu_{max}$  refers to maximum growth day (A-1). Then, A on the y-axis refers to the asymptotic maximum of  $\ln X_t / X_0$ . Biological parameters are important to determine the quality of water. There are factors that affect biological. In this case, algae are the factors that may affect quality of water.

### 2.3.4 Baranyi-Roberts Model

A new recruit of the growth model family described by Baranyi et al. (1993), in which a single variable describes the physiological status of the cells, is included. Assuming that the real growth rate immediately follow changes in the environment, the model may also explain bacterial growth in an environment where variables such as temperature and pH change over time is elaborated as follows by the first order of differential expression:

$$\mu(t) = \frac{1dx}{xdt} = \mu_{max} \alpha(t) f(t) \quad (5)$$

Where  $B(t)$  depicts the modification element in a bioreactor for inoculum customisation. In the following set, the modification element  $B(t)$  has a recurrent increase of  $0 \leq B(t) \leq 1$  and  $\lim_{t \rightarrow \infty} B(t) = 1$  based to

$$\alpha(t) = \frac{P(t)}{P(t) + Kp} = \frac{q(t)}{1 + q(t)} = \frac{q_0}{q_0 + e^{-\mu_{max} \cdot t}} \quad (6)$$

The quotient,  $q_0$ , applies to the inoculum's physiological state.  $P(t)$ , the cell adaptation mechanism to new surroundings was poor as the amount of essential material was assumed. Meanwhile, based on Michaelis-Menten kinetics,  $\mu_{max}$  was influenced by  $P(t)$ . In (7)