Predictive Growth Model of Indigenous Green Microalgae (Scenedesmus sp. UKM9) in Palm Oil Mill Effluent (POME)

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Abstract. Microalgae cultivation in POME treatment to removed environmental pollutants synchronized with biomass production. Biomass production depends on the proliferation rate of microalgae in wastewater, which is rarely addressed. Thus, growth rate assessment was performed on a local strain of Scenedesmus sp. UKM9 by using four different mathematical models of logistic, modified logistic, modified Gompertz, and Baranyi-Roberts. Statistical analyses, including root-mean-square error (RMSE), \( R^2 \), adjusted \( R^2 \), bias factor (BF), accuracy factor (AF), and standard error prediction (%SEP) were applied to verify the accuracy of each model. The findings illustrated that the Baranyi-Roberts model had the highest AF of 1.000, the lowest RMSE and %SEP values of 0.1300 and 5.40%, respectively, and the predicted BF value was 1.0001. These results revealed that the model was relevant to complex environmental conditions, and its parameter was biologically interpretable. The Baranyi-Roberts model regards a suitable model for the growth of indigenous microalgae of Scenedesmus sp. UKM9 in POME.

1. Introduction
Palm oil mill effluent (POME) is a non-toxic waste material obtained from the oil palm industry, which is also characterised as a viscous and acidic brown liquid with high nutrients load [1]. Recently, POME has received considerable attention as a microalgae cultivation medium. This is due to its nutrient richness and suitability as a scenario for the synchronised effluents remediation and biomass generation [2 - 5]. Furthermore, this culture approach is a viable method to manage the cost for microalgae biomass production by reducing the need for a costly synthetic growth medium. Researchers have recently shown increasing interest in indigenous microalgae with significant advantages due to their availability in the local ecosystem at low cost [6]. Notably, predicting the most appropriate growth model is crucial to understand the behaviour and potential of the microalgae under different environments. Scenedesmus sp. is a green microalgae consisting of unicells or flat coenobia colonies of two, four, eight, or sixteen linearly arranged cells. It has also been widely used in various industries, such as biofuel production, plastic formulation, and wastewater management due to its efficient growth and adaptation to environmental conditions [7 - 9]. There is a wide range of sizes and amounts of cells in coenobia, especially in water bodies with environmental conditions, including pH, nutrients, and temperature. This
growth modelling of microorganisms could be employed to determine the state of organisms in a wide range of environmental conditions [10].

There has been a prevalent use of the mathematical model for the prediction of cell development by estimating the maximum specific growth rate, lag phase, and maximum cell concentration. The model also enables the predictions of microorganism development, optimised development conditions, and the security and condition of the microalgae in different environmental conditions. As reported, mathematical models are highly prominent due to the successful interpretation of growth data based on field data [11]. For the growth of microalgae in wastewater, researchers have applied non-linear models to demonstrate development curves and specific compositional features of microalgae [12]. Three-factor models, including the modified Gompertz and Logistics models, were some of the models with the highest frequency of use due to specific biological factors, including lag time (λ), particular growth rate (μ), and asymptotic value (A) [12-15]. However, this model did not demonstrate the growth performance of microalgae in intense wastewater. Furthermore, Lam et al. [12] applied three kinetic models (Richards, Gompertz, and Logistic models) to estimate the development of *Chlorella vulgaris*. The continuous addition of domestic wastewater to the microalgae cultivation led to an insufficient curve fitting where the residual plots fluctuated and deviated from the X-axis.

Other studies displayed similar results of the impact of concentrated wastewater on the predictive growth model of microalgae [13, 16]. Accordingly, this study attempts to predict the appropriate growth model for local microalgae *Scenedesmus* sp. UKM9 in POME based on a four-parameter model (Baranyi-Roberts model). The achieved results were compared with the results of the three-parameter model (modified logistic model, modified Gompertz model) and the empirical model (logistic model). This was followed by the evaluation of the similarities and differences based on microalgal growth profiles and the significance of statistical analysis.

2. Materials and method

2.1 Source of cultivation media

Anaerobic POME was used as a microalgae cultivation media. It was collected from a clarifier overflow after an anaerobic digester of palm oil mill located at Bestari Jaya, Selangor, Malaysia. After collected, anaerobic POME was stored at 4°C. Prior cultivation, anaerobic POME was centrifuged at 8000 rpm for 10 minutes to ensure that the suspended solid was removed from the POME sample. The characteristics of anaerobic POME before and after pre-treatment shown in Table 1.

| Parameter                        | Anaerobic POME | Anaerobic POME after pre-treatment |
|----------------------------------|----------------|-----------------------------------|
| Chemical oxygen demand (COD)     | 14100 ±5264    | 2811±125                          |
| Total nitrogen (TN)              | 810±110        | 268±60                            |
| Total phosphate (TP)             | 340±94         | 190±70                            |
| pH                               | 8.0 ±0.05      | 8.0 ±0.05                         |
| Turbidity (NTU)                  | 748±179        | 257±17                            |

2.2 Microalgae cultivation

The experiments were performed with a local isolated green microalgae *Scenedesmus* sp. UKM9 with NCBI number: KU170547. These species were isolated from the wastewater of the Batu Anam rubber processing plant, Segamat Johor, Malaysia. The stock culture was maintained in Bold Basal medium (BBM), while the ingredients were prepared according to Ding et al. [16]. The seed culture was cultivated in a 1000 mL glass bottle, which contained 700 mL of BBM with an additional 5% v/v of POME. The intention of adding 5% (v/v) POME to BBM is to allow the adaptation of microalgae to the new environment (POME). A cool white fluorescent light was constantly illuminated on the media, which was then aerated with compressed air at ambient temperature. A sequel investigation using the batch cultures was performed with 700 mL of POME in 1000mL of the Duran bottle for thirty days.
20% (v/v) of the adapted Scenedesmus sp. UKM9 seed was transferred to the POME media and monitored for growth performance. Throughout the cultivations, the growth of microalgae in POME was quantified using the gravimetric method [17]. Additionally, the examinations were performed in triplicates, with the POME medium being managed without the microalgae as a control.

2.3 Mathematical model and statistical analysis

Four mathematical models, including logistic, modified logistic, modified Gompertz and Baranyi-Roberts were employed. The fitting procedure was performed using MATLAB R2020a through the Levenberg-Marquardt algorithm.

2.3.1 Logistic Model

The logistic model is an empirical model generated by Pearl and Reed [18] following the view made by Verhulst. This model demonstrated that the population growth started at an exponential rate and slowed down when the maximum population was reached. The functions of this model were expressed as follows:

\[ y = \frac{A+C}{1+e^{B(t-M)}} \]  

where, A was asymptotic at \( \frac{X_{t}}{X_{0}} \) as with the constant reduction of t, while C was asymptotic at \( \frac{X_{t}}{X_{0}} \) with the regular rise of t, B was the development rate at time M (day^{-1}), t indicated time (day), and M was the point of the highest complete development degree (day). Furthermore, \( X_{t} \) referred to the microalgal biomass concentration at time t (g L^{-1}), while \( X_{0} \) was the original concentration of microalgal mass (g L^{-1}).

2.3.2 Modified Logistic Model

This model is mechanistic originated from the measurement of a logistic empirical model, where the terms related to the microbiological meaning of the phase lag (\( \lambda \)) and maximum growth rate (\( \mu_{\text{max}} \)) were incorporated into mathematical parameters [19] based on the following equation:

\[ y = \frac{A}{A + \exp^{-B(t-M)}} \]  

where, \( \lambda \) referred to the lag phase (day), while \( \mu_{\text{max}} \) was the maximum growth rate (day^{-1}), and A was the asymptotic ln \( \frac{X_{t}}{X_{0}} \) maximum on the y-axis.

2.3.3 Modified Gompertz model

This model is a mechanistic model adjusted by Zwietering et al. [20] from the Gompertz model, which also consists of biologically important parameters, including the lag phase and maximum growth rate. The equation is as follows:

\[ y = A\exp\left\{-\exp\left[\frac{\mu_{\text{max}}A}{A}(\lambda - t) + 1\right]\right\} \]  

where, \( \lambda \) was the lag phase (day), while \( \mu_{\text{max}} \) represented the maximum growth rate (day^{-1}), and A referred to the asymptotic ln \( \frac{X_{t}}{X_{0}} \) maximum on the y-axis.

2.3.4 Baranyi-Roberts Model

Although the Baranyi model is mechanistic, it is geometrically different as it represents the quasilinear segment at the exponential phase. Baranyi et al. [21 - 22] proposed that cell population diversity over time could be elaborated through the first order of differential expression:

\[ \mu(t) = \frac{1}{x} \frac{dx}{dt} = \mu_{\text{max}} \alpha(t) f(t) \]  

where, \( \alpha(t) \) represented the adjustment function, which indicated the inoculum customisation in the bioreactor. The adjustment function \( \alpha(t) \) had a regular increase, as represented by \( 0 \leq \alpha(t) \leq 1 \) and \( \lim_{t \to \infty} \alpha(t) = 1 \) according to the equation below:
\[ \alpha(t) = \frac{p(t)}{P(t) + K_p} = \frac{q(t)}{1 + q(t)} = \frac{q_0}{q_0 + e^{-\mu_{\text{max}} t}} \] (5)

The quotient \( q_0 \) referred to the physiological condition of the inoculum. As it was following the volume of the critical substance, \( P(t) \), it slowed down the procedures of cell adaptation to their new environment. Meanwhile, \( \mu_{\text{max}} \) was affected by \( P(t) \) based on Michaelis–Menten kinetics. Moreover, the \( f(t) \) function in Eq. (4) indicated the restricted end-of-growth, which regularly reduced with \( f(0) = 1 \) and \( \lim_{t \to \infty} f(t) = 0 \). Most dynamic models demonstrated \( f(t) \) using a logistic inhibition, as illustrated in the following equation:

\[ f(t) = 1 - \left( \frac{x}{X_{\text{max}}} \right) \] (6)

After rearranging Eqs. (4)-(6) Baranyi [24] proposed the equation as:

\[
y = A + \mu_{\text{max}} x + \frac{1}{\mu_{\text{max}}} \ln\left( e^{-\mu_{\text{max}} x} + e^{-h_0} - e^{-\mu_{\text{max}} x - h_0} \right) - \ln\left( 1 + e^{\mu_{\text{max}} x + \frac{1}{\mu_{\text{max}}} \ln\left( e^{\mu_{\text{max}} x - h_0} - e^{\mu_{\text{max}} x + h_0} \right) - 1} \right)
\] (7)

where, \( y \) referred to \( \ln \left( \frac{X_t}{X_0} \right) \), while \( \mu_{\text{max}} \) was the highest development rate (day\(^{-1}\)), \( A \) was the original cell concentration (\( X_0 \)), \( y_{\text{max}} \) referred to the asymptotic \( \ln \left( \frac{X_t}{X_0} \right) \) with the constant rise of \( t \). Furthermore, \( h_0 \) was a dimensionless parameter quantifying the original physiological condition of the cells. The calculation of the lag duration \( \lambda \) (day) could be represented as \( \frac{h_0}{\mu_{\text{max}}} \).

2.4 Statistical analysis

This analysis aims to evaluate the performance of four types of mathematical models by determining the statistical parameters, including regression coefficient \( (R^2) \), adjusted regression coefficient \( (R^2) \), bias factor \( (\text{BF}) \), root mean squared error \( (\text{RMSE}) \), standard error prediction \( (\%\text{SEP}) \), and accuracy factors \( (\text{AF}) \) according to the following mathematical and statistical equations:

\[
R^2 = \frac{\sum_{i=1}^{n}(y_{i,\text{ob}} - y_{i,\text{pd}})^2}{\sum_{i=1}^{n}(y_{i,\text{ob}} - \bar{y})^2} \] (8)

\[
\text{Adjusted } R^2 = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \] (9)

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(P_{i,\text{pd}} - P_{i,\text{ob}})^2}{n-p}} \] (10)

\[
\text{BF} = 10^{\frac{\sum_{i=1}^{n}\log\left(\frac{P_{i,\text{pd}}}{P_{i,\text{ob}}}\right)}{n}} \] (11)

\[
\text{AF} = 10^{\frac{\sum_{i=1}^{n} \left| \log\left(\frac{P_{i,\text{pd}}}{P_{i,\text{ob}}}\right) \right|}{n}} \] (12)

\[
\%\text{SEP} = \frac{100}{\text{mean}_{\text{obs}}} \times \left[ \frac{\sum_{i=1}^{n} (\text{obs}_{i} - \text{pred}_{i})^2}{n} \right]^\frac{1}{2} \] (13)

The statistical comparison between group was conducted by one-way ANOVA and Turkey post hoc test using GraphPad Prism 8.
3. Results and discussions

3.1 Growth curve assessment

Figure 1 shown the growth curve of the Scenedesmus sp. UKM9 strain in anaerobic POME for a month with a comparison of the control group. The biomass production pattern of this microalgae resembles a sigmoidal shape with a lag phase. The growth curve showed an increase in biomass of UKM9 isolates which is much faster than that the control group (anaerobic POME without inoculation of UKM9). Although POME without inoculation of Scenedesmus sp. UKM9 was used as a control, the slight biomass production was observed due to the presence of other indigenous bacteria in the POME. The maximum biomass concentration measured for the UKM 9 strain was 1.13g/L and for control group was 0.17g/L.

![Growth profile of Scenedesmus sp. UKM9 in anaerobic POME. Data represent the mean ± standard deviation of three replicates.](image)

3.2 Curve fitting assessment

Mathematical models are powerful tools, which are widely used to determine the behaviour of a system. In the microbiology industry, mathematical modelling is employed to estimate the cell growth trends by computing the maximum specified growth rate, lag phase, and maximum cell concentration. Figure 2 illustrates the curve fit of logistic, modified logistic, modified Gompertz, and Baranyi-Roberts models under the optimization states of Scenedesmus sp UKM9 in anaerobic POME. The data used for these curve fittings were extracted from Figure 1. Since the microalgae grow exponentially, Figure 2 was plotted with a logarithm of the relative concentration size, \( y = \ln(X/X_0) \) against time (day). The visual analysis of logistic and modified logistic models was found to be less consistent with the experimental results. The modified Gompertz and Baranyi-Roberts models were in line with the actual data, as confirmed by the residual plot (see Figure 3). This residual plot illustrated the contrast between the estimated and tested values. Notably, a proper residual plot should display a focused value on the x-axis and conceal display scattered data. The logistic and the modified logistic models illustrated high sigmoidal curve scattering along the x-axis, indicating the model erroneousness in the experimental data matching. Additionally, the Baranyi-Roberts and modified Gompertz models indicated that the plot was more focused on the x-axis compared to the other models.
Figure 2. Simulated growth model of *Scenedesmus* sp UKM9 in anaerobic POME (a) Logistic model (b) Modified logistic model (c) Modified Gompertz model (d) Baranyi-Roberts model.

Figure 3. Residual plots (a) Logistic model (b) Modified logistic model (c) Modified Gompertz model (d) Baranyi-Roberts model.

3.3 Statistical analysis
The goodness-of-fit or credibility of the four growth models evaluated by statistical analysis and results obtained are illustrated in Table 2. The data analysis demonstrated that the curve fit for the logistic and modified logistics models was less satisfactory as the regression coefficient ($R^2$) was lower than 95%. Thus, lower precision was found in the prediction of the growth data of *Scenedesmus* sp. UKM9 on the anaerobic POME. Meanwhile, Baranyi-Roberts and modified Gompertz models showed a regression coefficient ($R^2$) of over 95%, with the models demonstrated values of 98.3% and 96.5% respectively.
The value of regression coefficient (R²) is commonly used for linear regression models. In the case of nonlinear regression, the number of parameters between models is different. Therefore, the adoption of regression coefficient (R²) analysis does not readily provide comparable analysis. The adjusted R² was then used to evaluate the quality of non-linear models. The adjusted R² for the logistic model amounted to 0.9310, while the modified logistic model was 0.9363, modified Gompertz model was 0.9596, and Baranyi-Roberts model was 0.9787. Notably, only the adjusted Gompertz and Baranyi-Roberts models presented a modified correlation coefficient of higher than 95%.

Overall, these study findings were in line with the results recorded by other researchers [13, 16, 23]. Specifically, Ding et al. [16] employed the modified logistic model to evaluate the growth kinetics of *Chlamydomonas* sp at 12.5%, 16.7%, 25%, 50%, and 100% of v/v POME. However, the growth kinetics data for POME with a concentration of higher than 50% v/v were not recorded as the value of μₘₐₓ was estimated to be too small. The model data was also less fitted with experimental data (R² < 95%). Furthermore, *Botryococcus braunii* growth kinetics data demonstrated a negative specific growth rate with a high concentration of POME (50% v/v) [23]. Meanwhile, the analysis by Razali et al. presented a small value of μₘₐₓ and R² < 95% when *Chlorella* sp was cultivated with a high concentration of POME [13]. Therefore, the results indicated that the logistic and modified logistic models were not feasible for the prediction of a microalgae growth model at a high concentration of POME.

Besides, Pearl and Reed [18] stated that the logistic model was more suitable for the simulation of growth population that increase by several fold, consistent with its original purpose to predict the dynamics of the human population. This model function tends to accumulate towards the final value of the biomass (Xₘₐₓ) and achieved at a low level (<1 g/L). Another probable drawback of it was the middle part of the exponent, which had an inflexion point in the sigmoid curve. This feature was not suitable for the description of the exponential growth phase due to the linear relationship between the logarithm of biomass concentration and time. Therefore, the sigmoidal type models could lead to an overestimated value of μₘₐₓ and λ [24]. In confirming this finding, the credibility of all prediction models was further evaluated through additional statistical analysis.

The details of regression parameters, such as root mean squared error (RMSE), bias factor (BF), accuracy factor (AF) and standard error prediction (%SEP), were determined. The RMSE presented an assessment of the relationship between the experimental and model data, with the ideally matching models exhibiting low RMSE values. Subsequently, the logistic, modified logistic, modified Gompertz, and Baranyi-Roberts models exhibited RMSE values of 0.2339, 0.2247, 0.1790, and 0.1300, respectively. It was found that Baranyi-Roberts model presented better RMSE value. Furthermore, the BF was calculated to compute the relative separation between the model and the observed value. The ideal match between the data from model predictions and experimental data were represented by the equivalent BF value of 1. Meanwhile, the BF values of higher and lower than 1 indicated that predicted data higher or lower than the tested data. As the prediction model presented a BF value ranging from 0.973 to 1.006, it considered as a good prediction model. While the BF value ranging from 0.7 to 0.9 or 1.06 to 1.15 was considered acceptable, it was rejected when the range amounted to < 0.7 or > 1.15 [25].

It was shown from Table 1 that the Baranyi-Roberts model produced the most ideal curve fit with a BF value of 1.0001. The accuracy AF indicated the mean contrast between model prediction and test data. As an effect of this increased element, a low volume was obtained for the estimation of correctness between the predicted and actual amounts. The amount presented in the Baranyi-Roberts model was the minimum AF values (1.0001), followed by the adjusted Gompertz (1.0018), modified logistic (1.0021), and logistic (1.0028) models. Additionally, the %SEP proved that the Baranyi-Roberts model demonstrated lower residuals with a difference of 5.04% between the predictive model and experimental values.
The statistical analysis of the models demonstrated that the Baranyi-Roberts and modified Gompertz models were the most ideal for the description of data regarding the growth of indigenous microalgae *Scenedesmus* sp. UKM9 in the POME media. Therefore, the evaluation of the features of each model is important for the selection of the most suitable prediction model. The selection criteria are based on the significance of each parameter in the model. The Baranyi-Roberts model parameters were found to be more mechanistic and have a biological meaning due to the presence of the four parameters (A, **Y**\(_{\text{max}}\), **μ**\(_{\text{max}}\), h\(_0\)) instead of three parameters (A, **μ**\(_{\text{max}}\), λ) in the modified Gompertz model. Parameter h\(_0\) in Baranyi-Roberts model was identified as the indicator of the adaptation of the population of microorganisms to the real environment [21]. The standardisation of the experimental procedure would allow the adaptation indicator to reach the constant value, creating an inversely proportional lag phase and **μ**\(_{\text{max}}\) assumptions.

The growth parameter values provided by Baranyi-Roberts models were A (0.0552), followed by **μ**\(_{\text{max}}\) (0.1426 d\(^{-1}\)), h\(_0\) (-0.2597), and **Y**\(_{\text{max}}\) (1.156 gL\(^{-1}\)) (see Table 3). A one-way ANOVA was performed to compare the **μ**\(_{\text{max}}\) of Baranyi-roberts, modified Gompertz and modified logistic models and the finding showed a significant result (p < 0.001). Furthermore, a post hoc comparative studies using the Tukey test showed that the mean score for the Baranyi-roberts model (Mean = 0.1426, Standard deviation = 0.001) was significantly different from Modified Gompertz model (Mean = 0.1963, Standard deviation = 0.004) and modified logistic model (Mean = 0.1866, Standard deviation = 0.004). However, no significantly difference of **μ**\(_{\text{max}}\) between modified Gompertz and modified logistic model. This suggested that **μ**\(_{\text{max}}\) value predicted by modified Gompertz and modified logistic models was more than expected. The lag phase obtained from Baranyi-Roberts model was also significant compare to modified logistic and modified logistic models (p<0.001). This finding is in line with results reported by other publications in the literature [24]. Baty and Delignette-Muller [24] found that Baranyi model gave best estimated and reasonable value of lag phase compared with the modified logistic and modified Gompertz models.

Based on the empirical data and statistical accuracy, the Baranyi-Roberts model was adequate for the presentation of the data on the growth of *Scenedesmus* sp UKM9 in the POME media. Moreover, it has been proven in the literature study that this model has been successfully used to model the algae growth in synthetic media and wastewater [26-28]. Therefore, the Baranyi-Roberts model was selected to predict the growth of *Scenedesmus* sp UKM9 in POME.

**Table 2.** Data on statistical model validation

| Model                  | n  | R\(^2\) | Adjusted R\(^2\) | RMSE | Bias factor (BF) | Accuracy factor (AF) | %SEP  |
|------------------------|----|---------|------------------|------|-----------------|----------------------|-------|
| Baranyi-Roberts        | 4  | 0.9829  | 0.9787           | 0.1300 | 1.0001         | 1.0001              | 5.40  |
| Modified Gompertz      | 3  | 0.9650  | 0.9596           | 0.1790 | 0.9962         | 1.0018               | 7.74  |
| Modified logistic      | 3  | 0.9448  | 0.9363           | 0.2247 | 0.9939         | 1.0021               | 9.72  |
| Logistic               | 3  | 0.9448  | 0.9310           | 0.2339 | 0.9902         | 1.0028               | 12.85 |

The kinetics data of *Scenedesmus* sp UKM9 with different predicted growth model

| Model              | n  | A      | λ      | **μ**\(_{\text{max}}\) | h\(_0\) | **Y**\(_{\text{max}}\) | B | C | M |
|--------------------|----|--------|--------|-----------------------|--------|----------------------|---|---|---|
| Baranyi Roberts    | 4  | 0.0552 | -1.82a | 0.1426                | -0.2597 | 1.156                | - | - | - |
| Modified Gompertz  | 3  | 2.8500 | -0.99b | 0.1963                | -       | -                    | - | - | - |
| Modified logistic  | 3  | 2.7790 | -1.09b | 0.1866                | -       | -                    | - | - | - |
| Logistic           | 3  | 11.9400 | -    |                      | 0.264c  | 9.157                | - | - | - |

(Different letters indicate significant differences between group using one-way ANOVA and post hoc Turkey test)
4. Conclusion
The current trends have proven that POME has the potential to be employed as an alternative element for microalgae growth. However, the prediction of microalgae growth in POME was found to be challenging for assessment due to its natural characteristics. Therefore, through the four models investigated in this study, it was found that the Baranyi-Roberts model was the most appropriate model employed to describe the growth of microalgae in POME, as illustrated from the study of empirical and statistical analysis.

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