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Growth Kinetics Determination Using Different Mathematical Models for Microalgae *Characium* sp. UKM1, *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4

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Microalgae are extensively used in industry due to their potential in producing high-value metabolites. The good microalgae growth kinetics performance is essential owing to excellent microalgae biomass harvesting efficiency. Therefore, the best mathematical model for the growth kinetics of microalgae is required to predict the correct growth kinetics value and helps in the elucidation of downstream processes. This study embarks on the objective to determine the best mathematical models for three local microalgae which are *Characium* sp. UKM1, *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4 cultured in Bold Basal Media (BBM). The four mathematical models are used to evaluate the growth kinetics of microalgae which include logistic model (Lm), modified logistic model (MLm), modified Gompertz model (MGm) and Baranyi-Roberts model (BRm). The experimental data were compared to the predicted data through the residual plot. The comparison shows that BRm is the best model to fit UKM1, UKM2 and UKM4 due to the experimental data which is close to the x-axis of the residual plot indicating the data were fitted the best to the BRm. The statistical analysis confirmed that all microalgae growth patterns exhibited that the BRm is the best model owing to the lowest percentage of standard error prediction indicating the lowest error compared to the other models. In addition, accuracy and bias factors are near to one which assess the precision of these models. In conclusion, the growth of UKM1, UKM2 and UKM4 grown in BBM is best fitted to the Baranyi-Roberts model.

Keywords: Microalgae; *Characium* sp. UKM1; *Chlorella* sp. UKM2; *Coelastrella* sp. UKM4; mathematical model

I. INTRODUCTION

Microalgae are widely used in wastewater treatment, CO_2 sequestration and the metabolites produced in microalgae are often used to produce third-generation biofuel. Microalgae can grow in a robust condition with faster growth rates compared to terrestrial plants. Nevertheless, high-value microalgae-based products are interesting insights for commercialisation (Japar, Takriff & Mohd Yasin, 2021).

In order to enhance the product accumulation in microalgae biomass, the growth of microalgae is an

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important parameter. The growth of microalgae requires several phases. The time for the microalgae to adapt to the new culture condition is represented as the lag phase (λ). The time taken for microalgae growth utilising substrate in the fermenter is called an exponential phase. In a given period, the growth accelerates to a maximal value of a specific growth rate (μmax). Next, the microalgae enter a stationary phase where the amount of growth is similar to death. Finally, the growth rate decreases and in the end reaches zero or an asymptote (A) (Johari, 2014).

The specific growth rate (μ) and lag phase (λ) of microbial growth was described by mathematical kinetic modelling. Despite the decline phase in the microbial growth profile, microalgae often used sigmoidal function to describe their growth. The growth phases usually resulted in a sigmoidal curve (López *et al.,* 2004). A sigmoidal growth data set can be described as a nonlinear regression model. The estimated μmax, λ and A can be derived from the growth model (Matsuda & Sugawara, 2017). Several sigmoidal functions describe microalgae growth such as Gompertz, modified Gompertz, logistic, modified logistic, Richards, Von Bertanlanffy, Baranyi-Roberts, Morgan and Weibull (López *et al.,* 2004). The growth of commercial microalgae species were scientifically evaluated. However, no studies have been carried out to understand the best mathematical model for local microalgae species, Characium sp. UKM1, *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4.

Characium sp. UKM1, *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4 are often used in the phycoremediation of palm oil mill effluent (POME) and CO_2 sequestration study (Ding *et. al.,* 2020; Minhat *et al.,* 2016). The study of microalgae growth during phycoremediation process is essential in the determination of their performance in harsh environmental conditions and high organic load. However, the suitability of a mathematical model to describe these microalgae curve fitting was not yet understood. Perhaps the comparison of different mathematical models to fit each of the experimental data is timely.

The logistic model is frequently used to describe microbial population growth. The changes in the number of organisms will be described by this model as the function of growth rate (μ), initial biomass (X_o) and maximum biomass concentration (X_{max}) with respect to the cultivation time (Phukoetphim *et al.,* 2017). The modified logistic model (MLm) is derived from the classical logistic (Lm) differential equation (Windarto, Eridani & Purwati, 2018). In this model, the estimated yield and lag phase could be obtained over time (Halil, 2020).

Modified Gompertz (MGm) is the model that has been widely used by researchers. During the stationary phase, it gives lag time, specific growth rate and maximum biovolume (Çelekli, Balci & Bozkurt, 2008). Meanwhile, the Baranyi model has become the most commonly preferred growth model owing to its excellent fitting potential. Therefore, this model is the best to understand various environmental conditions due to its ability to interpret various kinetics values (Yilmaz, 2011).

The best mathematical model has been used extensively to predict the growth kinetics including lag time (λ), potential values of maximum cell concentration (X_{max}), maximum specific growth rate (μmax) to estimate the trend of cell growth. Based on the best model, the correct growth kinetics value can be determined. The utilisation of incorrect mathematical models will create erroneous data and justification in downstream processes. Therefore, the comparison of different mathematical models will be evaluated in this study. The experimental data of *Characium* sp. UKM1, *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4 cultured in the Bold Basal Medium will be used to fit on different models of Lm (Fujikawa, Kai & Morozumi, 2003), MLm, MGm and BRm (Matsuda & Sugawara, 2017). In addition, the significant growth fit of microalgae will be indicated by statistical analysis.

II. MATERIALS AND METHODS

A. Microalgae Cultivation and Biomass Determination

The three native microalgae *Characium* sp. UKM1 (NCBI: KJ143753), *Chlorella* sp. UKM2 (NCBI: KP262476) and *Coelastrella* sp. UKM4 (NCBI: KP691597) were cultured in Bold Basal Media (BBM). Each culture was seeded with 10% (v/v) inoculum in 1000 mL of BBM in Duran bottle at a temperature of 25˚C under constant illumination with fluorescent light aerated with 0.5 vvm of air sparging (Ding *et al.,* 2020).

BBM content was prepared according to Hariz and Takriff (2017). Microalgal growth was evaluated for twenty days by determination of its biomass amount. The biomass was measured by using the dry cell weight method according to Hariz *et al.* (2018).

B. Mathematical Models and Growth Kinetics Evaluation

Four mathematical models were employed to compare the best-fit growth model for three native microalgae. The four models are Lm, MLm, MGm and BRm. MATLAB R2020a software was used for the growth rate fitting. Table 1 shows the equation used for each model. Each equation was fitted in the MATLAB R2020a for growth kinetics evaluation.

Table 1. Mathematical models equation used in this study

No.	Model	Equation
(1)	Logistic (Lm)	$y=\frac{A+C}{1+exp^{(-B(t-M))}}$
(2)	Modified Logistic (MLm)	$y=\frac{A}{1+expexp\left[\frac{4\mu max}{A}(\lambda-t)+2\right]}$
(3)	Modified Gompertz (MGm)	$y= Aexp\{-exp\left[\frac{\mu e}{A}(\lambda-t)+1\right]\}$
(4)	Baranyi - Roberts (BRm)	$y = A + \mu max\, x + \frac{1}{\mu max} ln\, ln\, (e^{-\mu x} + e^{-ho} - e^{-\mu x-ho}) - \ln\left(1 + \frac{e^{\mu x+\frac{1}{\mu max}lnln\left(e^{\mu x}+e^{-ho}-e^{-\mu x-ho}\right)-1}}{e^{(ymax-A)}}\right)$

According to Mohd, Yasin & Takriff 2021, in Lm, A represent as asymptotic at X_t /X_o as with the constant reduction of t, while C was asymptotic at X_t/X_o with the regular rise of t, B was the microalgae development rate at time M (day^{-1}), t indicated time (day), and M was the point of the highest complete development degree (day). Furthermore, Xt referred to the microalgae biomass concentration at time t (gL^{-1}), while X_o was the original concentration of microalgae mass (gL^{-1}).

In MLm and MGm, λ refers to the lag phase (day), while μ_{max} is the maximum growth rate (day^{-1}) and A is the asymptotic ln X_t/X_o maximum on the y-axis.

Meanwhile, in BRm, y referred to ln (X_t/X_o), while μ_{max} was the highest development rate (day^{-1}), A was the original cell concentration (X_o), y_{max} referred to the asymptotic ln (X_t/X_o) with the constant rise of t. Furthermore, h_o was a dimensionless parameter quantifying the original physiological condition of the cells. The calculation of the lag duration λ (day) could be represented as h_o / μ_{max} (Mohd, Yasin & Takriff 2021).

C. Statistical Analysis

In order to find the best model among four types of mathematical models, several statistical parameters were used. In this study, statistical parameters that have been used are regression coefficient (R^2), adjusted regression coefficient (R^2), bias factor (BF), root mean squared error (RMSE), sum square error (SSE), standard error prediction (%SEP) and accuracy factor (AF) according to the following mathematical and statistical equations in Table 2.

Table 2. The equations used for statistical analysis

No.	Equation
(5)	$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_{i_{obs}} - y_{i_{calc}})^2}{\sum_{i=1}^{n}(y_{i_{obs}} - \bar{y})^2}$
(6)	$Adjusted\,(R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)}$
(7)	$\mathrm{RMSE} = \sqrt{\frac{\Sigma(obs-pred)^2}{n}},$
(8)	$\mathrm{SSE} = \frac{1}{N}\sum_{i=1}^{N}(Y_{ei} - Y_{ci})^2$
(9)	$B_f = 10^{\left(\frac{\Sigma \log(pred/obs)}{n}\right)},$
(10)	$A_f = 10^{\left(\frac{\Sigma \lvert\log(pred/obs)\rvert}{n}\right)},$

(11) $$\%\text{SEP} = \frac{100}{meanobs}\sqrt{\frac{\Sigma(obs - pred)^2}{n}},$$

The predicted value by the model represents '*pred*' and the experimental data represent as '*obs*', the number of experimental data represented as '*n*' (Johari, 2014). Otherwise, Y_{ei} is the experimental value and Y_{ci} is the predicted value of Y_{ei} (Vega *et al.,* 2007), *N* is the number observation (Abbaszadeh *et al.,* 2011).

III. RESULTS AND DISCUSSION

A. Growth Curve Assessment of UKM1, UKM2 and UKM4

Figure 1 shows the growth curve of UKM1, UKM2 and UKM4 grown in BBM as observed for 20 days. Production of biomass trend of this microalgae follows the sigmoid curve with lag phase. The maximum biomass concentrations produced by UKM1, UKM2 and UKM4 are 0.3 gL^{-1}, 1.42 gL^{-1} and 1.74 gL^{-1}, respectively.

The cultivation parameters for the UKM1 are similar to UKM2 and UKM4. However, the growth for UKM1 is slower than UKM2 and UKM4. This shows that the conditions of this study are not suitable to enhance the growth of UKM1. The previous study shows that UKM1 can achieve maximum biomass up to 2.27 g/L (Minhat *et al.,* 2016). This is due to the presence of carbon dioxide (CO_2) during the cultivation of UKM1 as indicated in Table 3. It was shown that the maximum specific growth rate (μmax) of UKM1 can achieve up to 0.5625 day^{-1} in the experiment with the presence of CO_2 (Minhat *et. al.,* 2016; Khalid *et al.,* 2019), which is 3 to 4 fold higher compared to other experiments without CO_2 supplementation (Tamil Selvam, Pegnanathan & Takriff, 2015; Khalid *et al.,* 2019). This indicates that UKM1 is dependent on CO_2 as a carbon source for its growth.

Table 3. Comparison with previous study for the growth kinetics of *Characium* sp. UKM1

Microalgae sp.	Medium and Condition	Cultivation Day (days)	Xmax (g/L)	Umax (days-1)	Reference
Characium sp. UKM1	BBM	20	0.3	0.2741	This study
Characium sp. UKM1	BBM + 0.04% CO2	10	2.27	0.5625	(Minhat *et al.,* 2016)
Characium sp. UKM1	2 L, POME (10% inoculum)	20	-	0.163	(Tamil Selvam, Renganathan & Takriff 2015)
Characium sp. UKM1 (CR)	BBM + 5% CO2 (12:12 light: dark, 20% inoculation)	11	0.315	0.5	(Khalid *et al.,* 2019)
Characium sp. UKM1 (CR)	Pome + 20% distilled water	20	1.43	0.17	(Khalid *et al.,* 2019)

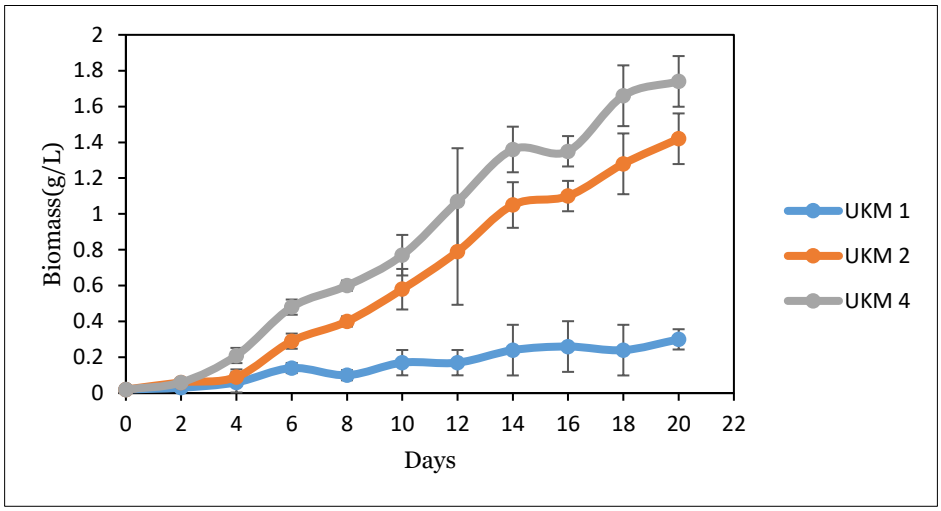

Figure 1. Growth curve of UKM1, UKM2 and UKM4 in BBM for 20 days. The data were represented as mean ± standard deviation of duplicated analysis.

The growth of microalgae as illustrated in Figure 1 were used to fit the four mathematical models: Lm, MLm, MGm and BRm. The curves fitted by four mathematical models for UKM1, UKM2 and UKM4 through MATLAB are presented in Figures 2, 3 and 4, respectively.

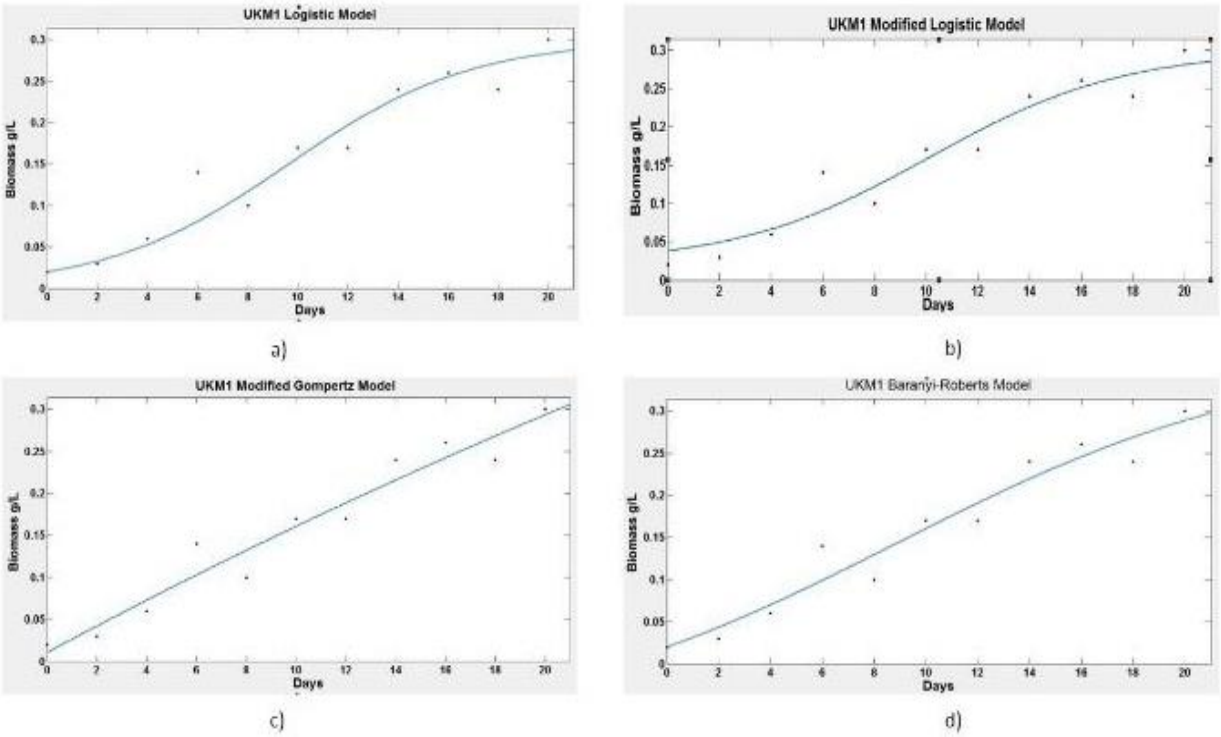

Figure 2. Mathematical model plots for UKM1; (a) Lm, (b) MLm, (c) MGm and (d) BRm

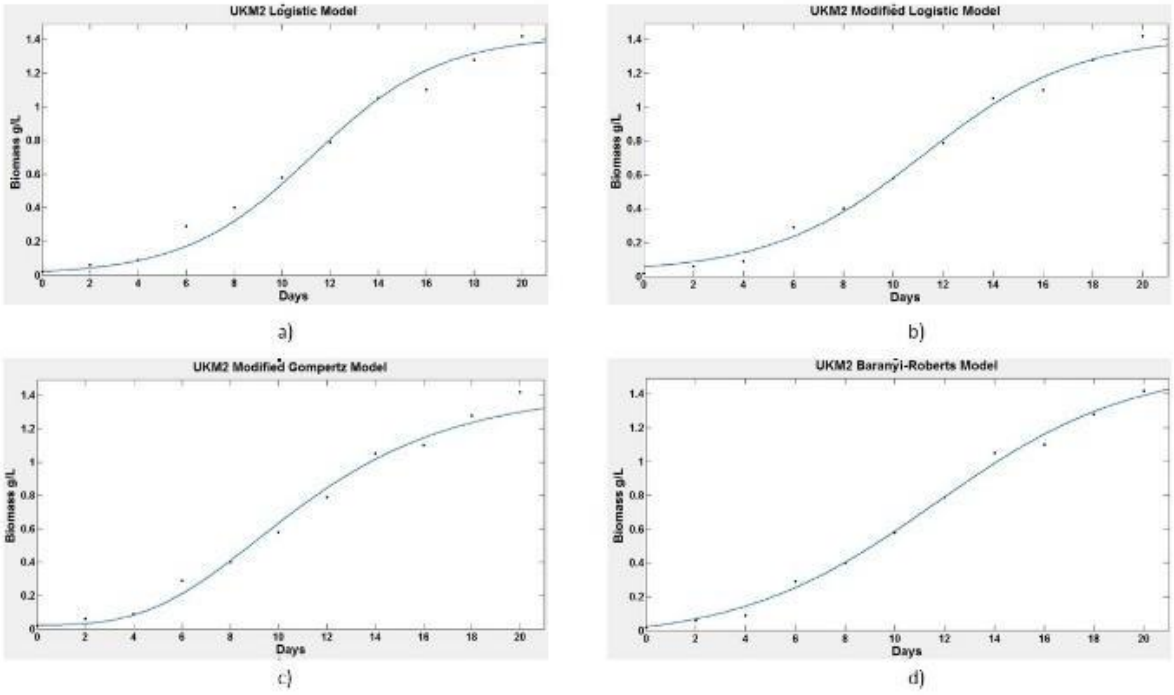

Figure 3. Mathematical model plots for UKM2; (a) Lm, (b) MLm, (c) MGm and (d) BRm

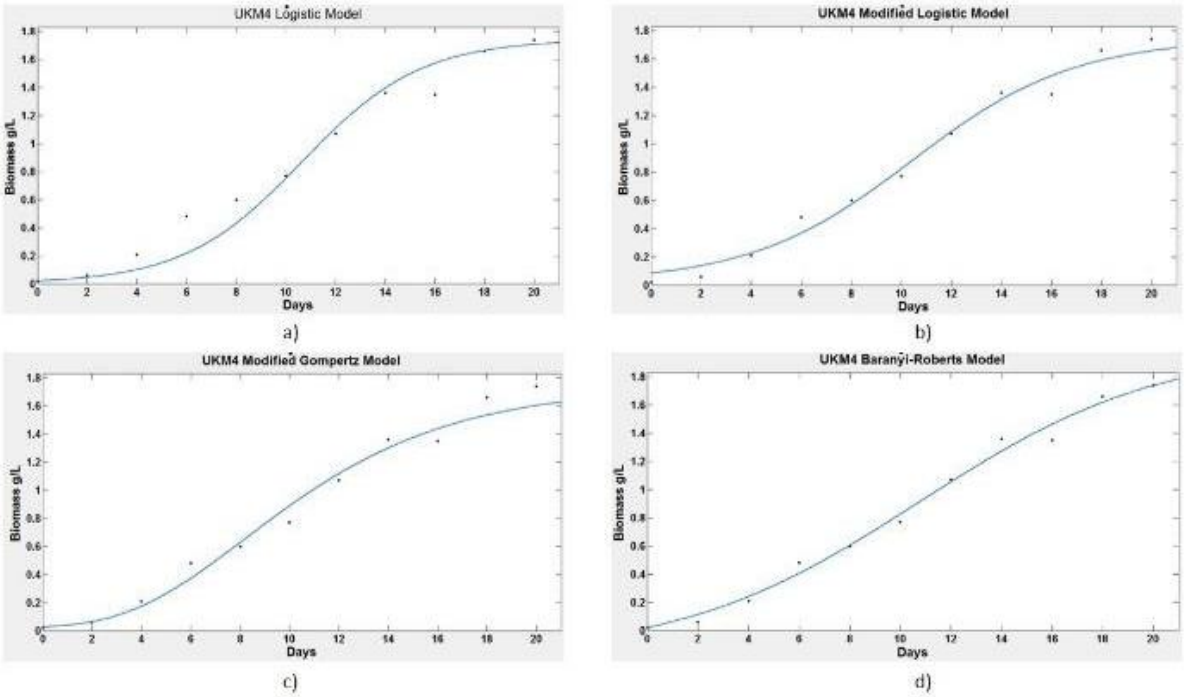

Figure 4. Mathematical model plots for UKM4; (a) Lm, (b) MLm, (c) MGm and (d) BRm

In general, all the models were fitted to all microalgae growth profiles, indicating the suitability of these four models in determination of the kinetics profile in microalgae. The mathematical model has been used extensively to predict the trend of cell growth by estimating the maximum specific growth rate, lag phase and maximum cell concentration (Lam *et al.*, 2017).

The best fit growth curve is when the plot is close to the x-axis. This to estimate the value of lag phase (λ) (Matsuda & Sugawara, 2017). In growth kinetics, this λ value is important. The best fit growth curve for UKM2 and UKM4 are Lm, MGm and BRm. However, all four mathematical models do not perform the best fit for the UKM1 growth curve. The model cannot fit well for UKM1 because the growth of UKM1 was not supported without the supplementation of CO_2 (Table 3). Thus, the growth pattern was not similar to UKM2 and UKM4.

However, the lag phase (λ) also can be obtained from the graph by drawing a straight line between the minimum and maximum exponent value, indicating the intersection to the x-axis is the lag phase value. The lag phase (λ) values are positive as indicated in Table 4. In growth kinetics, this λ value is important to estimate the time taken for microalgae to adapt to environmental culture conditions.

Finally, the growth rate decreases and in the end reaches zero or an asymptote (A). A previous study reported that the lag phase took about four days on average and the exponential phase took up about ten days for 10% of local microalgae cultured in BBM (Japar, Takriff & Mohd Yasin, 2021). In general, all the models were almost fitted to all microalgae growth profiles, indicating the suitability of these four models in determining kinetics profile in microalgae. However, further analysis needs to be carried out by looking at the differences between the experimental and predicted values.

Further analysis was carried out from the data illustrated in Figures 2, 3 and 4. The residual plot clarified the data as shown in Figures 5, 6 and 7 for UKM1, UKM2 and UKM4, respectively. The residual plot indicates the differences between the predicted and experimental values. The value must be closed to the x-axis and a random distribution pattern should not be displayed, indicating a good residual plot (Lam *et al.,* 2017).

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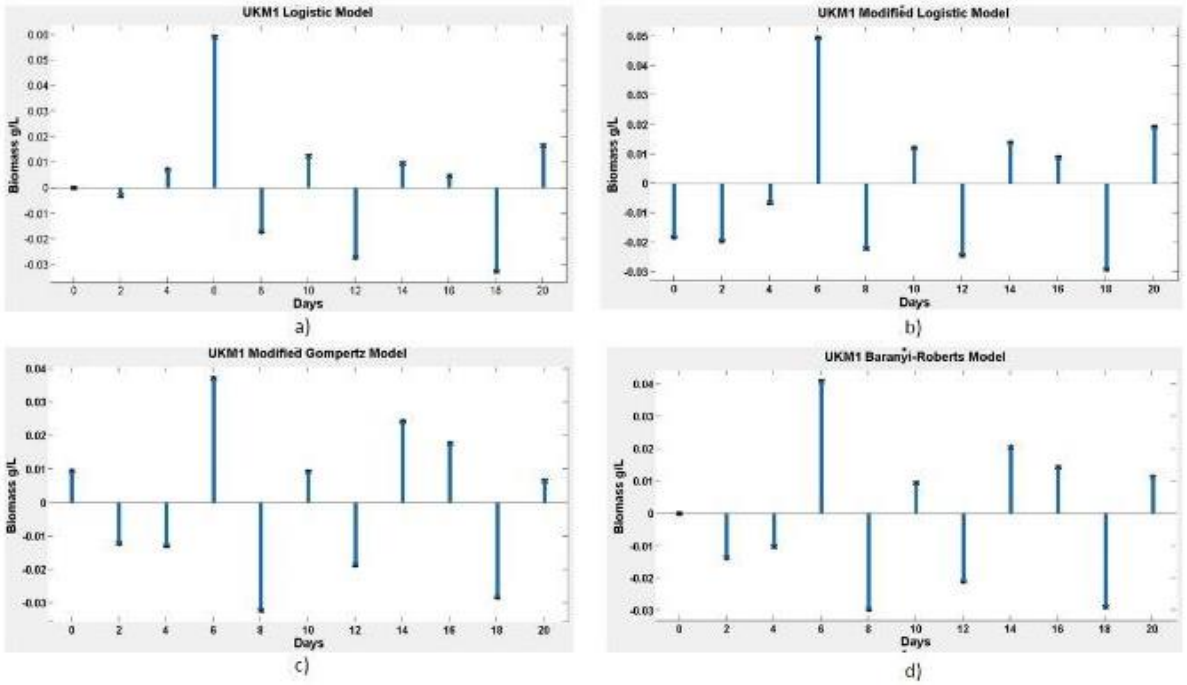

Figure 5. UKM1 residuals mathematical model plots; (a) Lm, (b) MLm, (c) MGm and (d) BRm

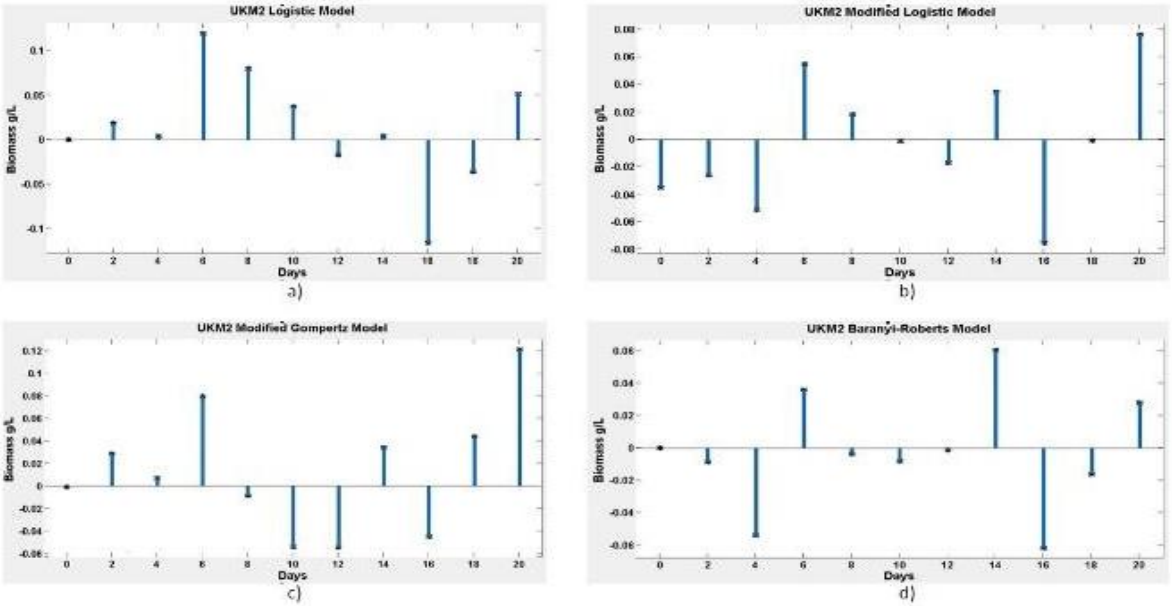

Figure 6. UKM2 residuals mathematical model plots; (a) Lm, (b) MLm, (c) MGm and (d) BRm

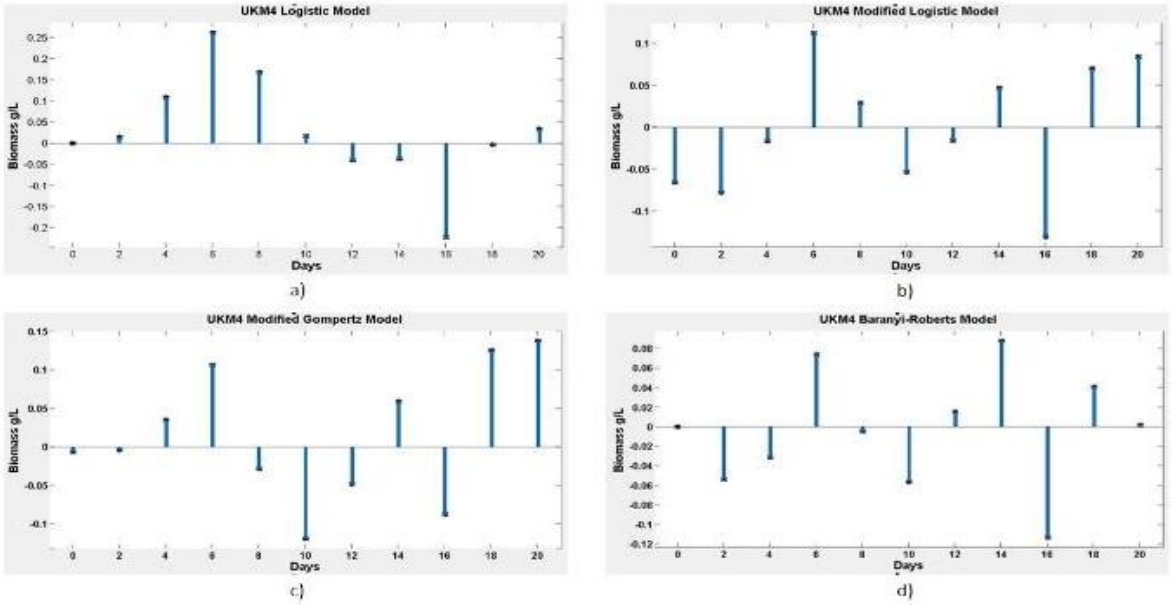

Figure 7. UKM4 residuals mathematical model plots; (a) Lm, (b) MLm, (c) MGm and (d) BRm

It can be observed from Figure 5(d), 6(d) and 7(d) that the best residual plot for UKM1, UKM2 and UKM4 is a BRm model due to the scattered trend with the least extent of sigmoidal bar placement along the x-axis. The other mathematical models proved that the models were not suitable for experimental data fitting due to the scattered trend with the greater extent of sigmoidal bar placement along the x-axis.

B. Statistical Analysis

To predict the best model for each microalga, the statistical analysis was carried out considering several factors such as regression coefficient (R^2), adjusted regression coefficient (R^2), bias factor (BF), root mean squared error (RMSE), sum square error (SSE), standard error prediction (%SEP) and accuracy factor (AF). The precision of each model in the curve fitting of the experimental data was indicated by the coefficient regression, R^2 (Lam *et al.,* 2017). Table 4 shows the several parameters of statistical analysis. This statistical analysis is to justify the best fit mathematical model

Table 4. Statistical analysis of mathematical models for three local microalgae species

	λ	μmax	R^2	adj R^2	SSE	RMSE	BF	AF	%SEP
UKM1									
Logistic model (Lm)		0.2741	0.9338	0.9338	0.0062	0.0248	0.9699	1.0311	15.05
Modified Logistic model (MLm)	2.541	0.0185	0.9369	0.9299	0.0059	0.0255	1.0977	1.0978	14.69
Modified Gompertz model (MGm)	1.964	0.0181	0.9392	0.9325	0.0057	0.0251	1.0414	1.0414	14.41
Baranyi-Roberts model (BRm)	9.505	0.1368	0.9469	0.9336	0.0050	0.0249	1.0404	1.0404	13.37
UKM2									
Logistic model (Lm)		0.3769	0.9849	0.9849	0.0397	0.0630	0.9038	1.1064	9.33
Modified Logistic model (MLm)	5.093	0.114	0.9921	0.9912	0.0208	0.0481	1.1536	1.1536	6.75
Modified Gompertz model (MGm)	4.598	0.1112	0.9875	0.9861	0.0329	0.0604	0.9166	1.0910	8.49
Baranyi-Roberts model (BRm)	8.957	0.2681	0.9951	0.9399	0.0129	0.0401	1.0448	1.0448	5.31
UKM4									
Logistic model (Lm)		0.4183	0.9586	0.9586	0.1628	0.1276	0.8377	1.1938	14.36
Modified Logistic model (MLm)	3.875	0.1333	0.9852	0.9835	0.0583	0.0805	1.2088	1.2088	8.60
Modified Gompertz model (MGm)	3.423	0.1299	0.9807	0.9786	0.0758	0.0917	1.0002	0.9998	9.79
Baranyi-Roberts model (BRm)	7.029	0.232	0.9911	0.9889	0.0349	0.0661	1.0619	1.0619	6.65

The values of more than 0.95 for R^2 indicate the accuracy of each model to fit with the experimental data. The curve fitting for the Lm, MLm, MGm and BRm model was unsatisfactory. It shows the value of less than 95% and lower precision was found as indicated in Table 4 for growth prediction of UKM1. It indicates that the highest R^2 for UKM2 and UKM4 are fitted to be BRm with the value of 0.9951 and 0.9911 for UKM2 and UKM4, respectively. According to the R^2 values, the result shows that the BRm model provides a good fit for UKM2 and UKM4.

However, the R^2 value is often used for linear regression models only. For nonlinear regression, the number of parameters expressed in the models would be different and R^2 analysis would not provide a comparative analysis. Therefore, the adjusted R^2 was further used for non-linear models quality evaluation (Johari, 2014).

The adjusted R^2 values for UKM1 to Lm, MLm, MGm and BRm model were 0.9338, 0.9299, 0.9325 and 0.9336, respectively. However, the value presented for UKM1 was unsatisfactory as the value of adjusted R^2 is less than 0.95 indicating lower precision in growth prediction. The adjusted R^2 shows that the MLm model gave higher precision in growth prediction in UKM2 with the value of 0.9912, while the BRm model was the highest precision for UKM4 with 0.9889 (Table 4).

Meanwhile, the lowest value of RMSE and SSE give the best fit (Abbaszadeh *et al.,* 2011). It was shown in Table 4 that the BRm presents an excellent model as reflected by lower RMSE and SSE value for all microalgae.

Then, to evaluate the relative difference between the predictive and observed values, BF was calculated. An ideal match between the mathematical model prediction data and experimental data can be represented by a BF value of 1. BF value of 1 shows the perfect agreement between the model with the experimental data (López *et al.,* 2004). The higher or lower BF values represent the overestimation or underestimation of the observed values, respectively. In this study, all four models produced the best prediction concerning the BF value. The perfect match between predicted and observed values is when the BF equals 1. The fail-dangerous model was indicated as the value of BF < 1, while the fail-safe model was indicated as the value of BF > 1 (Matsuda & Sugawara, 2017). The value can be considered acceptable for BF value in the range 0.70-0.90 or 1.06-1.15. However, the BF value that is considered unacceptable is in the range < 0.70 or > 1.15. Referring to this standard, there was no bias in this study for all models. All the BF values obtained in this study were within the good range (Dong *et al.,* 2007).

The mean contrast between the experimental and model prediction data presented as AF value. The typical AF value is more than or equal to 1. The higher AF value indicates insignificant efficiency of model prediction for correctness between the predicted and actual data (Matsuda & Sugawara, 2017). In this study, the Lm was found to be close to 1 for UKM1 with 1.0311. However, BRm was close to 1 with 1.0448 and 1.0619 for UKM2 and UKM4, respectively.

Furthermore, the lower error between the predictive and experimental values can be determined by the lowest value of %SEP. The %SEP proved that the BRm model demonstrated lower residuals with a difference of 13.8% for UKM1, 5.3% for UKM2 and 6.7% for UKM4 between the predictive and experimental values.

Statistical analysis shows that the ideal mathematical models are the BRm model for UKM1, UKM2 and UKM4 by referring to the lower value of %SEP. The BRm model present μmax= 0.1368 d^{-1}, λ= 9.505 and Xmax= 0.1546 gL^{-1} for UKM1. Meanwhile, the values for UKM2 is μmax= 0.2681 d^{-1}, λ= 8.957 and Xmax= 0.6305 gL^{-1}. In contrast, UKM4 give μmax= 0.232 d^{-1}, λ= 7.029 and Xmax= 0.8197 gL^{-1}. This is because most of the parameters by the BRm model are biologically interpretable (Johari, 2014).

A lower value of %SEP shows the lowest error in the models from all the statistical analysis parameters. Thus, the BRm model was selected due to its lower %SEP values for all three microalgae compared to the Lm, MLm and MGm models.

IV. CONCLUSION

The UKM1, UKM2 and UKM4 growth modelling were investigated using four different mathematical models. Logistic, modified logistic, modified Gompertz and Baranyi-Roberts models were used. All sigmoidal models can be used for the curve fitting of microalgae growth. However, based on the statistical evidence by comparing four models, it can

be concluded that the BRm model indicates the best mathematical model due to the lowest %SEP value. This presents that the BRm model has the lowest error compared to the others model. Therefore, the BRm is the best model to fit the microalgae growth for further kinetics studies.

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