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Mathematical Modelling of the Growth of *Burkholderia* sp. on Glyphosate

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ABSTRACT

Glyphosate is an agricultural herbicide with usage in the amounts of thousands of tonnes per year in Malaysia. In certain soils, glyphosate can persist for months and its removal through bioremediation is the most economical and practical. A glyphosate-degrading bacterium has been isolated from contaminated soil. The growth of the bacterium on glyphosate as the sole phosphorous source shows classical sigmoidal profile. Important growth constants can be reliably obtained through nonlinear regression modelling using growth models such as logistic, Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and the Huang models. The modified Gompertz model was chosen as the best model based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (adjR^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion). Novel constants obtained from the modelling exercise would be useful for further secondary modelling implicating the effect of media conditions and other factors on the growth of this bacterium on glyphosate. The fitted value of maximal growth rate showed a decline when the concentration of glyphosate was increased indicating substrate inhibition. The results from this work can be further used in secondary modelling exercises.

INTRODUCTION

Herbicides are primarily employed to wipe out and eradicate unwelcome terrestrial weeds. Nonetheless, the propensity of herbicides to be washed away especially during the rainy seasons led them to end up in the aquatic ecosystem. Aquatic plants and algae are subsequently the most susceptible group of aquatic non-target organis.ms. These plants aid in stabilising the sediments in lakes and running waters from breaking down. Glyphosate is a herbicide which targets the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme [1]. The enzyme transfers the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to 5-hydroxyl of shikimate-3-phosphate (S3P) through the shikimate pathway located in the chloroplast region [2].

Glyphosate affects organisms in the ecosystem through a variety of ways. For instance, fish and amphibians appear to have low sensibility towards glyphosate itself. The lethal dose LC_{50} was observed in channel catfish *Ictalurus punctatus* [3] ranged from 130 mg/L to 620 mg/L for carp *Cyprinus carpio* [4] in glyphosate treated water. Exposure to glyphosate on amphibians resulted to develop abnormalities. A high percentage of morphology alterations was observed in sharp-snouted tree frog (*Ssinax nassicus*) when incubated with 3 - 7 mg /L of glyphosate, which was the exact amount used in the sub-agricultural field. Uncontrolled usage aided by poor regulation by government strains the environment, as consequence more problems are emerging day by day.

As glyphosate tends to persist from weeks to several months in soil, their remediation is being highly researched. To date, bioremediation is the number one candidate for the remediation of this herbicide as bioremediation can deal with dilute concentration of target toxicants under complicated soil matrices, a feat where other approaches such as physicochemical methods will be uneconomic or ineffective.

Several studies were initiated to obtain bacterial strains with degrading ability to be used in biological treatment **[5]**. The initial discovery of isolates utilising glyphosate as a source of phosphate was *Pseudomonas* sp. PG2982 where it

metabolises glyphosate into sarcosine by clevaging the C-P bond instead of metabolising it into AMPA. Significant discovery has been also made in *Arthrobacter* sp. GLP-1 [6], *Alcaligenes* sp. GL [7], *Pseudomonas* sp. 4ASW [8], *Agrobacterium radiobacter* [9] and *Achromobacter* sp. MPS 12A [10] which utilises the same mechanism as *Pseudomonas* sp. PG2982. Meanwhile, *Flavobacterium* sp. GD1 [11], *Pseudomonas* sp. LBr [12], *Achromobacter* sp. LW9 [9], *Ochrobactrum anthropi* LBAA [13], *Ochrobactrum anthropi* GPK3 [10], *Ochrobactrum* sp. GDOS [14], *Bacillus subtilis* [15], *Klebsiella oxytoca* [16] and *Burkholderia* sp. AQ5–12 [17].

To date, *Bacillus subtilis* Bs-15 is one of the best glyphosate degraders with the maximum concentration tolerated by this bacterium reaches as high as 40,000 mg/L [18,19]. One of the keys aspect of glyphosate degradation by this bacterium is the growth on glyphosate exhibits a sigmoidal profile. This effect was not model according to available substrate inhibition models. Hence, the objective of this research is to model the degradation rate using nonlinear regression such as Luong, Yano, Teissier-Edward, Aiba, Haldane, Monod and Han and Levenspiel models [20–22]**.** Understanding the effect of glyphosate concentration on the degradation rate will significantly provide a crucial date for mass production and application of this strains in-situ contamination soils.

Callus growth, like bacterial growth is a linked process that displays unique phases where the specific growth rate, which initially has a value of zero producing a lag time (λ) then accelerates in a certain time period to a maximal value. The growth curves also include a final phase where an asymptote (*A*) is achieved where the rate gets to zero. Eventually, callus growth reaches a stage where the cells started to die and entering the death phase.

The overall profile of the growth rate appears sigmoidal curve [23]**.** One of the most important parameters of the growth curve is μmax (or μm). In biological systems, this value is used to develop secondary models such as the effects of product, pH, temperature, substrate on growth rate of the organism. The μmax or μm is usually given by the slope of the line at the exponential phase [24]. The most popular method in estimating this value is through conversion of the exponential phase to a linearized form usually via transforming the y values into logarithm or natural logarithm and then determining the slope of this curve using linear regression. A better method, but often neglected, is to model all of the set of data with nonlinear regression growth model and then getting the values of μmax, λ, and A from the model [25]

The modified Gompertz model is one of the classical growth models that include model such as the Verhulst [23,26]. The Gompertz function, named in 1844-1845 by Pierre François Verhulstis, is based on an exponential relationship between specific growth rate and population density. The initial stage of growth is approximately exponential; then, as saturation begins, the growth slows, and at maturity, growth stops. Gibson et al. [27] were the first to use the Gompertz equation to fit microbial growth curves and the equation was successfully used to describe the exponential and stationary phases of the microbial growth curves that is sigmoidal.

However, the model was not adequate to describe the lag phase. The model was modified by Gibson et al. [27] to incorporate the lag phase, and have been successfully used in modelling many microbial growth curves to the point where its dominance in mathematically modelling bacterial growth and product formation curves have been acknowledged [23,25,28].

Modelling of the growth curves can yield important parameters that can be used for further optimisation works for callus such as determination of specific growth rate, lag period and maximum callus *formation. In this study, the callus cultures from the seedling of Ficus deltoidea* was modelled according to the modified Gompertz model.

MATERIALS AND METHODS

Chemicals and media

Glyphosate PESTANAL® 99.5% analytical standard was purchased from Sigma, Aldrich USA. Meanwhile, other chemicals used in media were obtained from Fisher (Malaysia).

Growth and maintenance of bacterium

A previously isolated glyphosate-degrading bacterium [17] was maintained and grown on mineral salt medium with glyphosate as the sole phosphorus source. The composition of the medium in g/L of distilled water), pH (7.0 to 7.2) consists of: Tris buffer (1), glucose (5), NaCl (0.5), KCl (0.5) MgSO4.7H2O (0.2), NH4SO4 (2), CaCl2 (0.01) and FeSO4.7H2O (0.001) [29].

Fitting of the growth data

To decide whether there is a statistically substantial difference between models with different number of parameters, in terms of the quality of fit, data was statistically assessed through various methods such as the root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICc (Akaike Information Criterion) [30]**.**

RMSE

The RMSE was calculated according to Eq. (1), where *Pdi* are the values predicted by the model and Obi are the experimental data, n is the number of experimental data, and p is the number of parameters of the assessed model. It is expected that the model with the smaller number of parameters will give a smaller RMSE values (Eqn. 1).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(Pd_i - Ob_i\right)^2}{n-p}}$$

In linear regression models, the coefficient of determination or R^2 is used to assess the quality of fit of a model. However, in nonlinear regression where difference in the number of parameters between one model to another is normal, the adoption of the method does not readily provide comparable analysis. Hence, an adjusted R^2 is used to calculate the quality of nonlinear models according to the formula where RMS is Residual Mean Square and is the total variance of the y-variable (Eqns. 2 and 3).

$$Adjusted\ (R^2) = 1 - \frac{RMS}{s_Y^2} \quad (2)$$

$$Adjusted\ (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \quad (3)$$

Akaike information criterion with correction (AICc)

The Akaike information criterion (AIC) provides a means for model selection through measuring the relative quality of a given statistical model for a given set of experimental data [31]**.** AIC deals with the trade-off regarding the goodness of fit of the model along with the intricacy of the model. It is in reality founded on information theory. The procedure offers a comparative approximation of the information lost for every time a certain model is employed to signify the process that produces the information or data. For any output of a collection of predicted models, the most accepted model is the model demonstrating the minimum value for AIC. This value is often a negative value, with for example; an AICc value of -10 more preferred than the one with -1. The formula includes a number of parameters punishment, the greater the parameters, the less favoured the end result or the greater the AIC value. Therefore, AIC not simply returns goodness of fit, but additionally, doesn't really encourage utilizing more complex model (overfitting) for fitting experimental data. Considering that the data within this work is smaller compared to the number of parameter employed a remedied version of AIC, the Akaike information criterion (AIC) with correction or AICc is employed in its place. The AICc is computed for each and every data set for each model based on the following equation (Eqn. 4);

$$AICc = 2p + n\ln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad (4)$$

Where p is the number of parameters of the model and n is the number of data points. The procedure considers the alteration in goodness-of-fit and the improvement in number of parameters between two models. For each and every data set, the model having the smallest AICc value is extremely likely correct [31]**.**

Accuracy Factor (AF) and Bias Factor (BF)

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as recommended by Ross [32] were also employed. A Bias Factor equal to 1 indicates a perfect match between predicted and observed values. For microbial growth curves or degradation studies, a bias factor with values < 1 indicates a fail-dangerous model while a bias factor with values > 1indicates a fail-safe model. The Accuracy Factor is always ≥ 1, and higher AF values indicate less precise prediction (Eqns. 5 and 6).

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \quad (5)$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{|(Pd_i/Ob_i)|}{n}\right)}$$

RESULTS AND DISCUSSION

The bacterial growth on glyphosate from this bacterium was sigmoidal in shape and reaching maximum growth at approximately between 36 and 48 hours of incubation (Fig. 1). The growth profile over time was fitted to eight different models (**Table 1**). The resultant fitting shows visually acceptable fitting (**Fig. 1 to 7**). The modified Richards model failed to model the growth profile. The best performance was modified Gompertz model with the lowest value for RMSE, AICc and the highest value for adjusted R^2. The AF and BF values were also excellent for the model with their values were the closest to 1.0 (**Table 2**). The modified Gompertz model was then used to model the growth of the bacterium on various concentrations of phosphate (**Fig. 8**) and the growth constants obtained were then tabulated (**Table 3**).

Table 1. Growth models used in this study.

Model	p	Equation
Modified Logistic	3	$y = \frac{A}{\left\{1+\exp\left[\frac{4\mu_m}{A}(\lambda - t)+2\right]\right\}}$
Modified Gompertz	3	$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\}$
Modified Richards	4	$y = A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y = \left(\mu_m\frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y = A + \mu_m x + \frac{1}{\mu_m}\ln\left(e^{-\mu_m x} + e^{-h_0} - e^{-\mu_m x - h_0}\right) - \ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{\max}-A)}}\right)$
Von Bertalanffy	3	$y = K\left[1-\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(\mu_m x/3K^{\frac{1}{3}}\right)}\right]^3$
Huang	4	$y = A + y_{\max} - \ln\left(e^A + \left(e^{Y\max} - e^A\right)e^{-\mu_m B(x)}\right)$ $B(x) = x + \frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, IF X < LAG Y=A + K(X−λ), IF λ ≤ X ≥ X_{MAX} Y = Y_{MAX}, IF X ≥ X_{MAX}

Note:

a= bacterial lower asymptote;
μ_{MAX} = maximum specific growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
Y_{MAX} = bacterial upper asymptote;
e = exponent (2.718281828)
t = sampling time
α,β, k = curve fitting parameters
h_0 = a dimensionless parameter quantifying the initial physiological state of the cells. the lag time (day-1) can be calculated as $h_0=\mu_{MAX}$

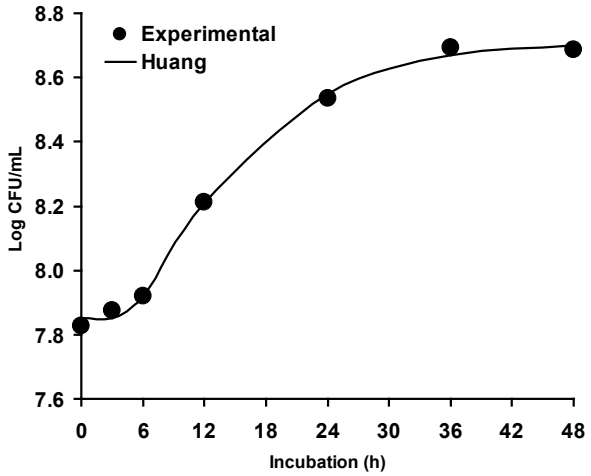

Fig. 1. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the Huang model.

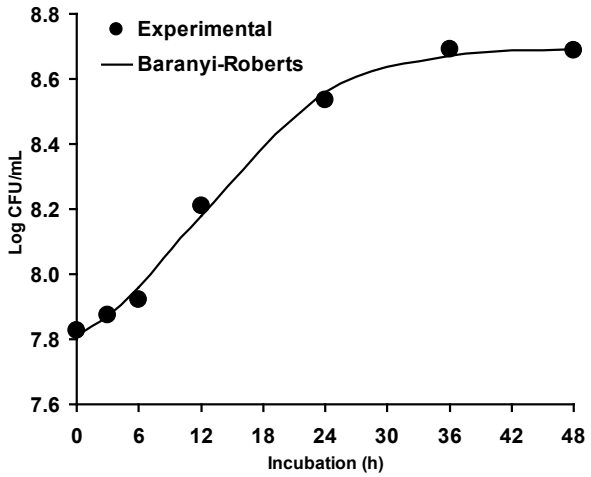

Fig. 2. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the Baranyi-Roberts model.

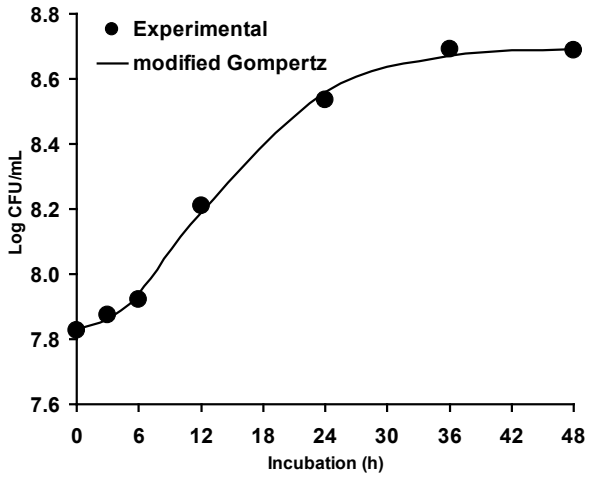

Fig. 3. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the modified Gompertz model.

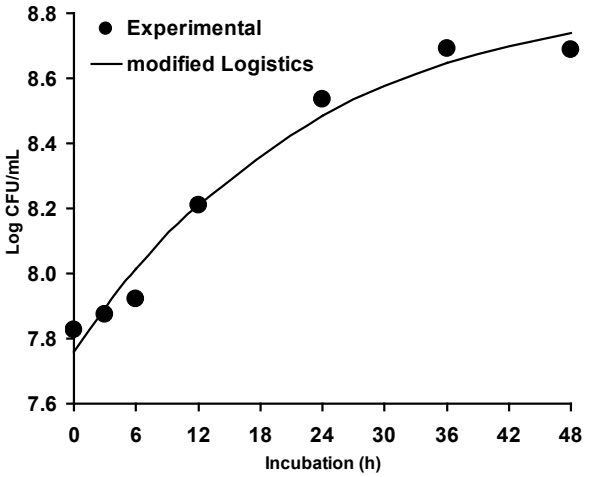

Fig. 4. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the modified Logistic model.

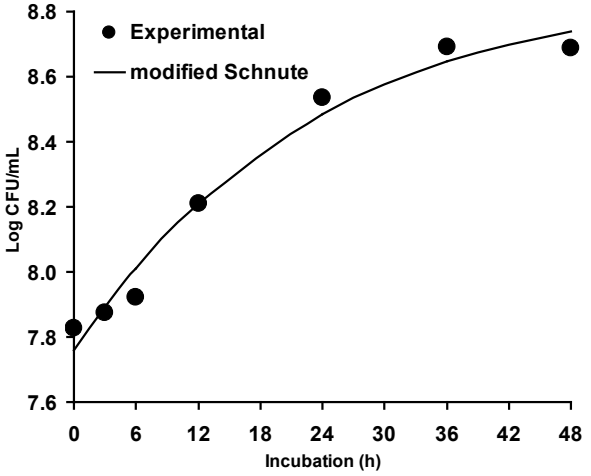

Fig. 5. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the modified Schnute model.

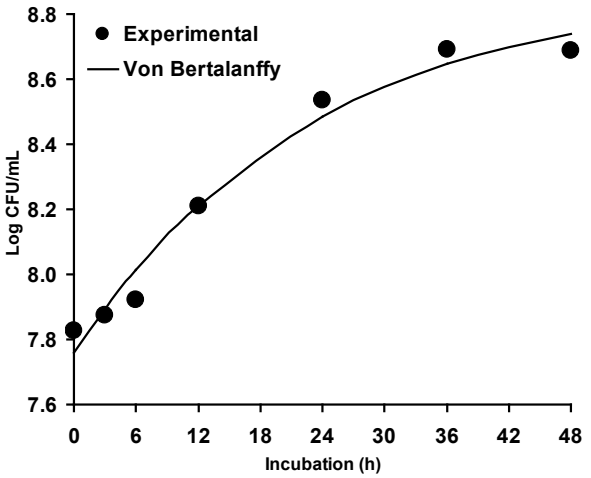

Fig. 6. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the Von Bertalanffy model.

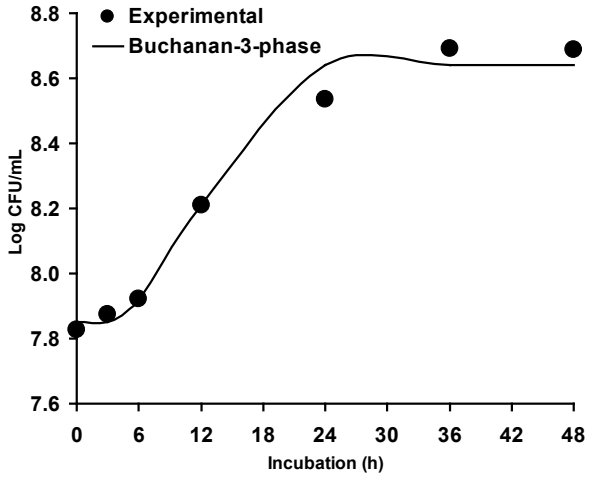

Fig. 7. The growth of *Burkholderia* sp. on 50 mg/L glyphosate as the sole phosphorous source fitted to the Buchanan-3-phase model.

Table 2. Statistical analysis of the various fitted models.

Model	*P*	RMSE	*AdjR*^{2}	AF	BF	AICC
Huang	4	0.03	0.99	1.00	1.00	20.64
Baranyi-Roberts	4	0.04	0.99	1.00	1.00	25.55
Modified Gompertz	3	0.02	1.00	1.00	1.00	-27.98
Buchanan-3-Phase	3	0.05	0.97	1.00	1.00	-11.13
Modified Richards	4	Nil	Nil	Nil	Nil	Nil
Modified Schnute	3	0.07	0.95	1.01	1.00	35.07
Modified Logistics	3	0.07	0.95	1.01	1.00	-6.79
Von Bertalanffy	4	0.07	0.95	1.01	1.00	-6.50

Note:
p no of parameters
adR^2 Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor
AICc Adjusted Akaike Information Criterion

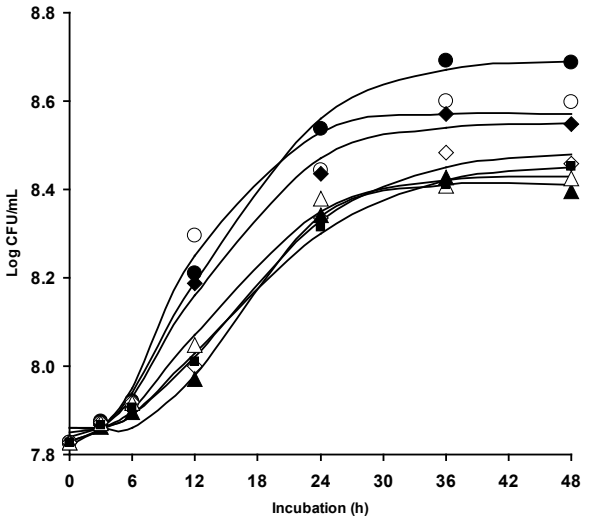

Fig. 8. The effect of glyphosate concentrations at 50 (●), 100 (○), 150 (◆), 200 (◇), 250 (▲), 300 (△) and 400 (■) mg/L on the growth of the bacterium fitted to the modified Gompertz model.

Table 3. Bacterial growth coefficients on glyphosate fitted to the modified Gompertz model. Values include standard error.

Glyphosate	μ_{Max} (H^{-1})	Lag (H)
50	0.044 ± 0.004	3.358 ± 1.474
100	0.053 ± 0.021	3.705 ± 2.67
150	0.041 ± 0.006	3.501 ± 1.938
200	0.041 ± 0.009	6.654 ± 2.192
250	0.034 ± 0.006	9.267 ± 1.307
300	0.031 ± 0.005	5.561 ± 1.637
400	0.027 ± 0.002	4.9 ± 1.368

The study carried out here attempt to optimize bacterial growth using mathematical models. Other growth models that are available including Baranyi-Roberts [33,34] and Logistic, modified Gompertz [35,36] b p. 600; [37–39,39–41], Richards, Schnute [23,27], Von Bertalanffy [42,43], Buchanan three-phase [38,44–49] and more recently the Huang model [50]. The modified Gompertz model is the most popular model as it is the simplest (having three parameters).

The asymmetrical sigmoidal shape of the modified Gompertz represents and may offer greater flexibility than the logistic. Sigmoidal models such as the logistic and Gompertz differ chiefly at the point of inflection between the lower and the upper asymptotes with the logistics and Gompertz models having the distance of 1/2 and 1/e between the lower and the upper asymptotes, respectively [28]. In an essence, other growth models provide flexible slope function and variable point of inflection between the lower and upper asymptotes. These functions are either special or simpler cases of a parent growth model. For instance the Richard model incorporates the logistics, Gompertz or von Bertalanffy growth models [23,27,28]. The model has its drawbacks and is not perfect with several main issues. Firstly, in the static version, y(t=0) is not equal to *yo*. Secondly, an inflection point is the intrinsic property of the sigmoidal curve causing the model to have a systematic problem in describing the exponential phase [33]. Finally, the model tend to over-estimates its parameter values [51–53]. Despite this, the modified Gompertz model has been extensively used to model the growth of bacteria and bacterial secondary products production such as biohydrogen, methane, lactic acid, biofuel and bacterioricin to name a few [54–58] including callus growth [39,59,60].

Parameters obtained from the fitting exercise would be later used for further secondary modelling. These mechanistic models are aimed to reach a better understanding of the chemical, physical, and biological processes. Compared to empirical model, mechanistic models including the modified Gompertz are more powerful since they tell you about the underlying mechanism or processes that drives the change in growth rates observed [61].

CONCLUSION

In this study, the effect various concentrations of glyphosate on bacterial growth profile has been successfully modeled using the modified Gompertz model. Parameters obtained from the fitting exercise shows substrate inhibition to the growth rate. This effect is bets modeled using various substrate inhibition models available and will be the subject of future studies.

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