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Mathematical Modelling of Glyphosate Degradation Rate by *Bacillus subtilis*

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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 previously isolated glyphosate-degrading bacterium showed substrate inhibition to the degradation rate. Important degradation inhibition constants can be reliably obtained through nonlinear regression modelling of the degradation rate profile using substrate inhibition models such as Luong, Yano, Teissier-Edward, Aiba, Haldane, Monod and Han and Levenspiel models. The Aiba 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) and accuracy factor (AF). The calculated values for the Aiba-Edwards constants q_{max} (the maximum specific substrate degradation rate (h^{-1}), K_s (concentration of substrate at the half maximal degradation rate (mg/L) and K_i (inhibition constant (mg/L)) were 131±34, 4446±2073, and 24323±5094, respectively. 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 degradation of glyphosate by this bacterium.

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 organisms. These plants aids 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] 1996) 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 (*Scinax nasicus*) 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 cleaving the C-P bond instead of metabolising it into AMPA. Significant discovery has been also made in *Arthrobacter* sp. GLP-1 (Pipke & Amrhein, 1988), *Alcaligenes* sp. GL [6], *Pseudomonas* sp. 4ASW [7], *Agrobacterium radiobacter* [8], and *Achromobacter* sp. MPS 12A (Sviridov *et al*., 2012) which utilises the same mechanism as *Pseudomonas* sp. PG2982. Meanwhile, *Flavobacterium* sp. GD1 (Balthazor & Hallas 1986), *Pseudomonas* sp. LBr (Jacob *et al*., 1988), *Achromobacter* sp. LW9 (McAuliffe *et al*., 1990), *Ochrobactrum anthropi* LBAA (Kishore & Barry 1992), *Ochrobactrum anthropi* GPK3 (Sviridov *et al*., 2012), *Ochrobactrum* sp. GDOS (Hadi *et al*., 2013), *Bacillus subtilis* [10], *Klebsiella oxytoca* [11] and *Burkholderia* sp. AQ5–12 [12].

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. One of the keys aspect of glyphosate degradation by this bacterium is the rate of degradation is severely inhibited at high concentrations of phosphate. 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 [13–15]. 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.

Table 1. Various mathematical models developed for reduction kinetics involving substrate inhibition.

Author	Degradation Rate	Author
Monod	$q_{\max}\dfrac{S}{K_s+S}$	[16]
Haldane	$q_{\max}\dfrac{S}{S+K_s+\dfrac{S^2}{K_i}}$	[17]
Teissier	$q_{\max}\left(1-\exp\left(-\dfrac{S}{K_i}\right)-\exp\left(\dfrac{S}{K_s}\right)\right)$	[18]
Aiba-Edward	$q_{\max}\dfrac{S}{K_s+S}\exp\left(\dfrac{-S}{K_i}\right)$	[18,19]
Yano and Koga	$\dfrac{q_{\max}S}{S+K_s+\left(\dfrac{S^2}{K_1}\right)\left(1+\dfrac{S}{K}\right)}$	[20]
Han and Levenspiel	$q=\dfrac{q_{\max}S\left[1-\left(\dfrac{S}{S_m}\right)\right]^n}{K_s+S-\left[1-\left(\dfrac{S}{S_m}\right)\right]^m}$	[21]
Luong	$q_{\max}\dfrac{S}{S+K_s}\left[1-\left(\dfrac{S}{S_m}\right)^n\right]$	[22]

Note:

q_{max}	maximal degradation rate (h^{-1})
K_s	half saturation constant for maximal reduction (mg/L)
S_m	maximal concentration of substrate tolerated and (mg/L)
m, n, K	curve parameters
S	substrate concentration (mg/L)

MATERIALS AND METHODS

Data acqusition

Graphical data of a published work [10] from Figure 2 (Effect of initial concentration of glyphosate to degradation rate) were electronically processed using WebPlotDigitizer 2.5 [23] which helps to digitize scanned plots into table of data with good precision and reliability [24,25].

Fitting of the data

The data were fitted using a nonlinear regression that uses a Marquardt algorithm CurveExpert Professional software (Version 1.6), which minimizes the sums of square of the differences between values of the predicted and measured.

Statistical analysis

Various statistical methods such as the root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF) and accuracy factor (AF) were utilized [26]. The root-mean-square error or RMSE was calculated according to **Eq. 1**, where p is the number of parameters of the assessed model, Ob_i are the experimental data, Pd_i are the values predicted by the model and n is the number of experimental data.

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

Calculations of the adjusted R^2 is carried out according to the following equations where RMS is Residual Mean Square and S_y^2 is the total variance of the y-variable.

$$Adjusted\ \left(R^2\right)=1-\frac{RMS}{s_Y^2} \quad \textbf{(Eqn. 2)}$$

$$Adjusted\ \left(R^2\right)=1-\frac{\left(1-R^2\right)(n-1)}{(n-p-1)} \quad \textbf{(Eqn. 3)}$$

Other tests for the goodness-of-fit of the models; Accuracy Factor (AF) and Bias Factor (BF), have been introduced by Ross [27]. The equations are as follows;

$$\text{Bias factor}=10^{\left(\sum_{i=1}^{n}\log\frac{\left(Pd_i/Ob_i\right)}{n}\right)} \quad \textbf{(Eqn. 4)}$$

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

RESULTS AND DISCUSSION

Statistical analysis (**Table 2)** shows that the best model was Aiba-Edwards with the majority of the statistical evaluation such as the lowest values for RMSE, the highest adjusted R^2 values and with Bias Factor and Accuracy Factor nearest to unity (1.0) indicated that the model was the best.

Table 2. Statistical analysis of kinetic models.

Model	p	RMSE	adR2	BF	AF
Luong	4	5.293	0.893	0.999	1.050
Yano	4	4.646	0.919	0.995	1.048
Tessier-Edward	3	nil	nil	nil	nil
Aiba-Edwards	3	4.159	0.951	0.997	1.049
Haldane	3	5.387	0.914	1.011	1.072
Monod	2	15.129	0.058	1.004	1.227
Han and Levenspiel	5	7.327	0.762	0.997	1.227

Note:

SSE	Sums of Squared Errors
RMSE	Root Mean Squared Error
P	No of parameters
R^2	Coefficient of Determination
adR^2	Adjusted Coefficient of Determination
BF	Bias Factor
AF	Accuracy Factor

Most of the models tested showed good fitting as observed by eye with the exception of the Monod model (**Figs 1 to 6**). The Teissier model failed to fit the experimental data. showThe calculated values for the Aiba-Edwards constants q_{max} (the maximum specific substrate degradation rate (h^{-1}), K_s (concentration of substrate at the half maximal degradation rate (mg/L) and K_i (inhibition constant (mg/L)) were 131±34, 4446±2073, and 24323±5094, respectively.

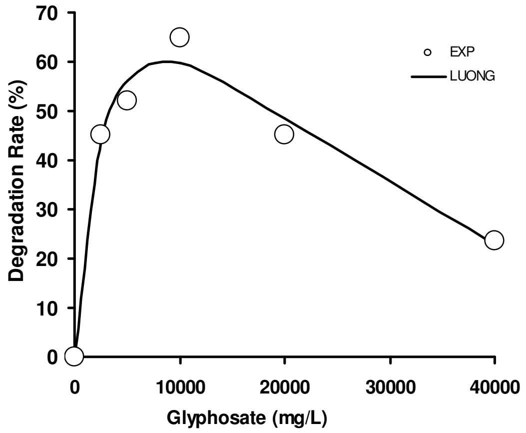

Fig. 1. Fitting the effect of glyphosate to glyphosate degradation rate experimental data with the Luong model.

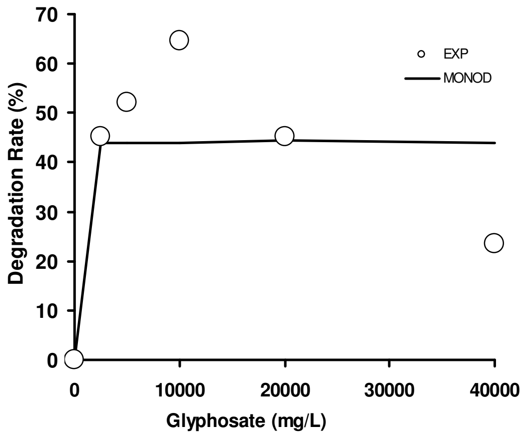

Fig. 2. Fitting the effect of glyphosate to glyphosate degradation rate experimental data with the Monod model.

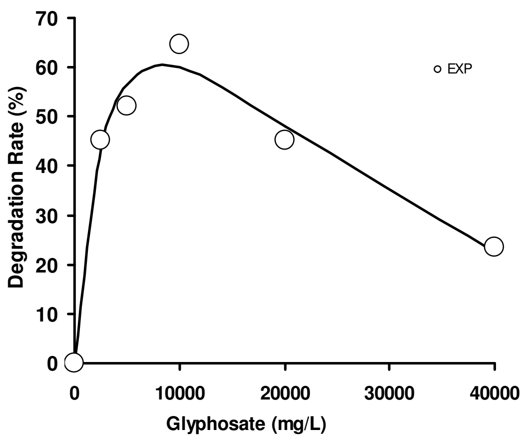

Fig. 3. Fitting the effect of glyphosate to glyphosate degradation rate experimental data with the Han-Levenspiel model.

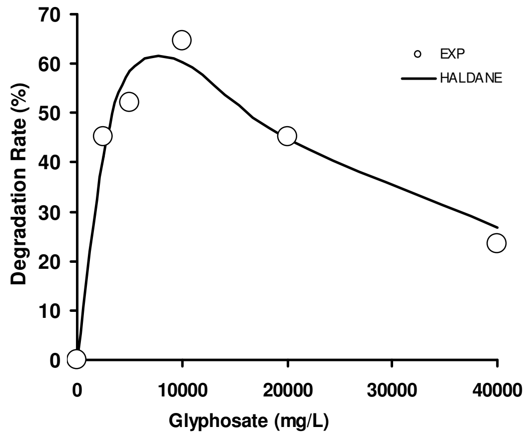

Fig. 4. Fitting the effect of glyphosate to glyphosate degradation rate experimental data with the Haldane model.

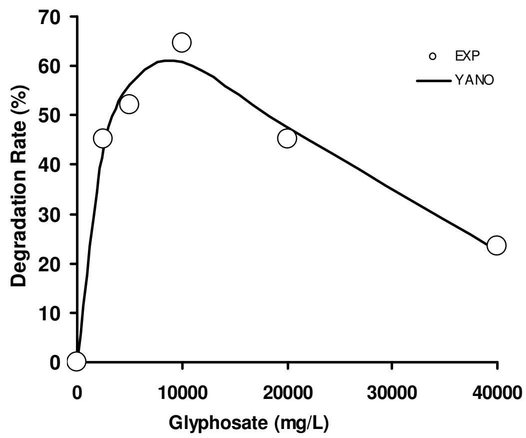

Fig. 5. Fitting the effect of glyphosate to glyphosate degradation rate experimental data with the Yano model.

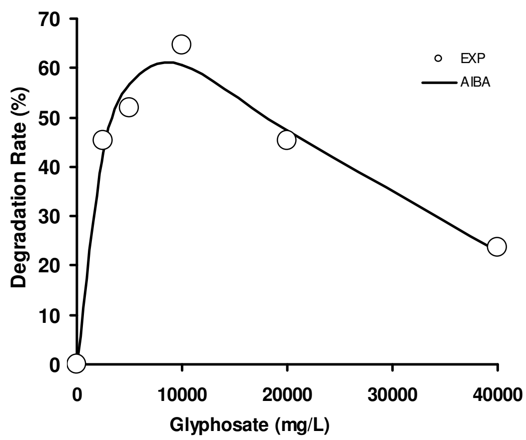

Fig. 6. Fitting the effect of glyphosate to glyphosate degradation rate experimental data with the Aiba model.

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. The Bias Factor equal to1 indicate 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.

Many xenobiotics biodegradation studies use substrates that inhibits microbial growth or substrate biodegradation due to the toxicity of the substrates. These substrates include aromatic and halogenated hydrocarbons and even elemental biotransformation processes that include metals such as mercury, chromium and molybdenum [28–30]. Under this circumstance the Monod model will fail to describe the degradation of growth profile and other models such as Wayman and Tseng [31], Haldane, Luong, Han-Levenspiel, Andrews and Noack, and Webb can be used [32]. Another model of inhibitory kinetics was proposed by Aiba et al. (1968) but it involves product inhibition kinetics. The model was subsequently modified to assume a common mechanism for both product and substrate inhibition to rate by Edwards (1970). This modified version (Aiba–Edwards model) has been reported to be the best model to fit phenol degradation by *Ralstonia eutropha* [33] and inhibitory effect of NaCl on the halotolerant *Kocuria rosea* [34]. The Aiba-Edwards model has not been able to model substrate inhibition kinetics for many xenobiotics but its usage for describing the effect of glyphosate concentration to glyphosate degradation rate in this work is novel.

CONCLUSION

The glyphosate degradation by a bacterium exhibited classical substrate inhibition to the degradation rate. This degradation kinetics of bacteria can be modelled using various models available in the literature. Of the numerous models to describe the effect of substrate to the degradation rate, the Aiba-Edwards model was the best based on statistical reasoning. This is the first time that the model was utilized to model the effect of substrate on glyphosate degradation rate.

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