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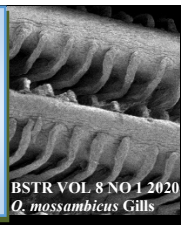

Growth Mathematical Modelling of the Effect of Cyanide on *Pseudomonas putida* (Naun-16)

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ABSTRACT

Gold mining companies have been known to use cyanide to extract gold from minerals. The indiscriminate use of cyanide presents a major issue in the environment. The used of linearisation methods using natural logarithm transformation is inaccurate, even though is standard and can just give an estimated value for the sole parameter measured; the specific growth rate. In this study, various cyanide concentrations ranging from 0 - 350 mg/L was used. Seven different mathematical models such as such as modified Logistics, modified Gompertz, modified Richards, modified Schnute, Baranyi-Roberts, Von Bertalanffy and most recent Huang were used to get values for the above constants or parameters from bacterial growth *Pseudomonas putida* on cyanide. The best model was found to be modified Logistics with the lowest AICc and RMSE value. The modified Logistics parameters such as Y_{max} (bacterial growth upper asymptote), λ (lag time), μ_{max} (maximum specific bacterial growth rate) and A or Y_0 (bacterial growth lower asymptote) were found to be 2.41 (95% confidence interval of 2.37 - 2.45), -3.16 (95% confidence interval of -4.64 to -1.68) and 0.12 (95% confidence interval of 0.11 to 0.13). This is the first report of growth mathematical modelling of the effect of cyanide on *Pseudomonas Putida* (Naun-16).

INTRODUCTION

Cyanide played a significant part in the evolution of life on Earth and it remains an essential nitrogen source for bacteria, plants and fungi. Though, some microorganisms can synthesise cyanide, a large number have the ability the degrade cyanide [1]. Plants are the major source of cyanide in the atmosphere since they cogenerate cyanide with ethylene in addition to producing cyanolipids and cyanoglycosides. Furthermore, cyanide has also been demonstrated to be produced as part of catalytic proteins active iron-cyanide complexes [2]. Cyanide is a very toxic compound that is released into the environment via the sewages of industrial activities such as mining, organic chemicals production, coal coking, photography, electronics and metal plating [3]. Because of high cyanide toxicity, it has the ability to kill the respiratory system by preventing the last step of electrons to oxygen from cytochrome C oxidase thereby preventing ATP production. Small quantity of cyanide exposure can be fatal regardless of the way of exposure [4]. More so, Cyanide waste is gradually becoming a rampant problem in today's society. Biological treatment, ozonation, hydrogen peroxide technique, alkaline chlorination is among the current treatment for cyanide pollution with biological treatment being the promising and latest in addition to its advantages over other conventional chemical techniques [5]. This technique is widely accepted by the public and regulatory agencies because it is relatively cheap since bacterial cells can be made active by simple aeration besides, it could absolutely degrade large amount of cyanide. This method could degrade cyanide without generating a new waste like slush or other byproducts and is friendly to the environment [6]. Dursun and Aksu [7] reported that these economical and promising reasons made the biological technique to become a more feasible alternative.

Several studies have shown the use of microorganisms such as bacteria, fungi, algae among others from the genera of *Rhodococcus*, *Pseudomanas*, *Serratia marcescens*, *Bacillus nealsonii*, *Alcaligenes* spp., *Burkhoderia cepacia*, *Acinetobacter* and *Streptomyces phaeoviridae* as the useful Actinobacteria for successful bioremediation of cyanide [3,8,9]. Others include

few yeast and algae like *Cryptococcus cyanovorans* sp. nov., Chlorella spp., *Scenedesmus obliquus* and *Arthrospira maxima* [10]. *Rhodococcus* UKMP-5 M previously isolated from hydrocarbon-polluted environment showed a great potential of biodegradation of cyanide to a non-toxic product [8]. Some microorganisms such as *Serratia marcescens* have been reported to degrade cyanide at acidic or neutral pH [9]. Ebbs [1] reported that these microorganisms use different enzymes like cyanide dioxygenase, cyanide oxygenase, cyanide dihydratase and cyanide hydratase in executing their functional processes following oxidative or hydrolytic reactions in carrying out these decontaminations, by converting cyanide to carbon dioxide, formate or ammonia.

Researches have shown that bacterial growth was sigmoidal in shape [11–13]. In order to describe the bacterial growth curve, various sigmoidal functions such as Von Bertalanffy, Baranyi-Roberts, modified Schnute, modified Richards, modified Gompertz, modified Logistics and stannard were compared [14]. They were compared statistically using a comprehensive model (Schnute model), which is a model that encompasses all other models. Furthermore, the models were compared with respect to their easy usage. In order to contain all biologically relevant parameters, all sigmoidal functions were modified. The models of Stannard, Schnute and Richards seemed to be essentially the same equation [15]. In the cases tested, the modified Gompertz equation was statistically adequate to explain the growth of cyanide data.

Cyanide degradation mathematical modelling was been previously reported [5] to use linearisation of the cyanide degradation over time profile to obtain the specific growth rate for further secondary modelling. As the benefits of nonlinear regression analysis of the cyanide have been described above, therefore, this study is aimed at evaluating several available models such as Huang model [16], Buchanan three-phase [17], Gompertz [14,18], Logistic [14,19], Richards (14,20), Schnute (14), Baranyi-Roberts [15] and Von Bertalanffy [21].

MATERIALS AND METHODS

Data from Fig 2a. from Singh et al [22] was processed using the software Web plot digitiser 2.5 [23] which digitises the scanned Figure and has been utilised by many researchers and acknowledged for its reliability [24].

Statistical analysis

Statistical significant difference between the models was calculated through various methods including the adjusted coefficient of determination (R2), accuracy factor (AF), bias factor (BF), Root-Mean-Square Error (RMSE) and corrected AICc (Akaike Information Criterion) as before [25,26].

Fitting of the data

Fitting of the bacterial growth curve using various growth models (**Table 1**) was carried out using the CurveExpert Professional software (Version 2.6.5) by nonlinear regression utilising the Marquardt algorithm. μ_{max} of estimation was carried out by the steepest ascent rifle of the curve while the crossing of this line with the x-axis is an estimation of λ [26,27].

Lastly, the last datum point is an estimation for the asymptote (A). The Huang's model needs to be solved mathematically as it is differential equation. The Runge-Kutta method was utilised through the ode45 solver in MATLAB (Version 7.10.0499, The MathWorks, Inc., Natick, MA).

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	$y=A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda-t\right.\right.$
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	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-\right.$ $\left.-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{max}-A)}}\right)\right)$
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 growth lower asymptote;
μ_{max}= maximum specific bacterial growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
y_{max}= Bacterial growth 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 reduction process. The lag time (h^{-1}) can be calculated as $h_0=\mu_{max}$

RESULTS AND DISCUSSION

Fig. 1 shows the result for cyanide bacterial growth curve of *Pseudomonas putida* at various concentrations of cyanide. The data shows that as cyanide concentration is increased, the bacterial growth decreased, hence, reaching an optimum concentration at 100 mg/L. Therefore, it could be concluded that cyanide has a substantial effect on the growth of the bacterial strain. At 350 mg/L cyanide, the bacterial growth is significantly decreased, showing the inhibitory effect of cyanide at higher concentration.

Based on the bacterial growth modelling (**Figs. 2-8**), the best model was found to be modified logistic model with the lowest value for RMSE, AICc and the highest value for adjusted R2. The AF and BF values were also excellent for the model with their values were the closest to 1.0. The poorest performance was the Huang model (**Table 3**). The coefficients for the modified logistic model are shown in **Table 2**.

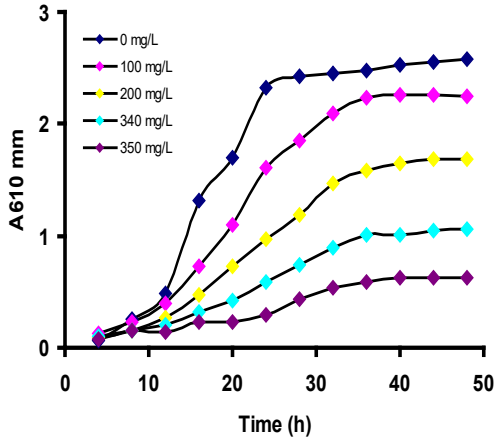

Fig 1. Growthof *Pseudomonas Putida* (Naun-16) In nutrient broth (Nb) supplemented with various concentrations of cyanide.

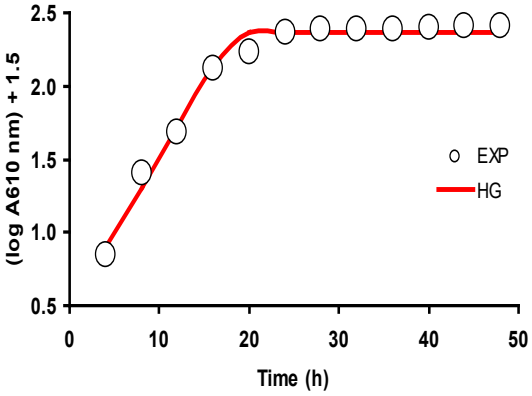

Fig. 2. Growth of *Pseudomonas Putida* (Naun-16) as modelled using the Huang model.

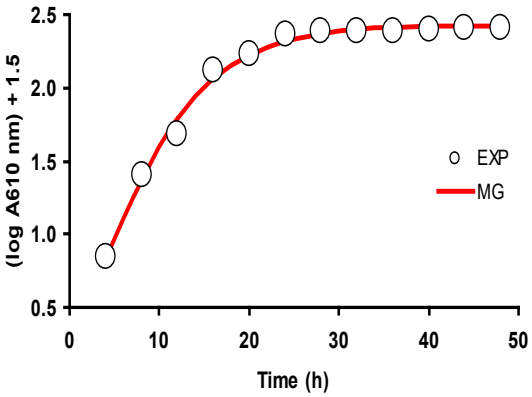

Fig. 3. Growth of *Pseudomonas Putida* (Naun-16) as modelled using the modified Gompertz model.

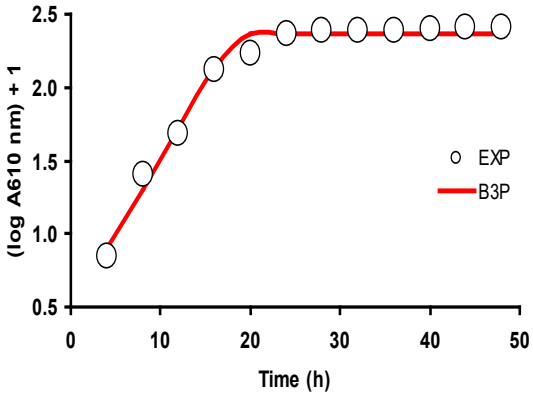

Fig. 4. Growth of *Pseudomonas Putida* (Naun-16) as modelled using the Buchanan-3-phase model.

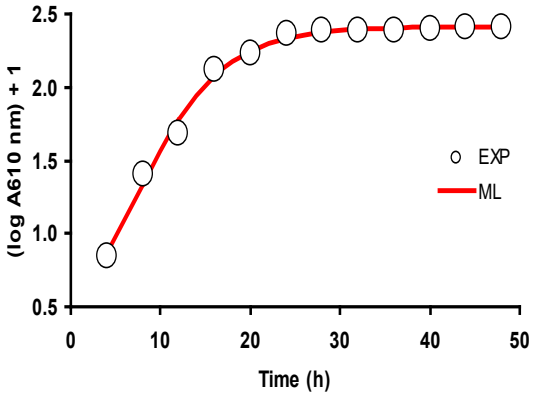

Fig. 5. Growth of *Pseudomonas Putida* (Naun-16) as modelled using the modified Richard model.

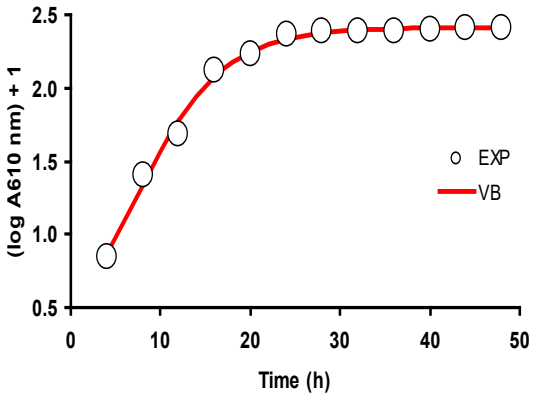

Fig. 6. Growth of *Pseudomonas Putida* (Naun-16) as modelled using the modified Logistics model.

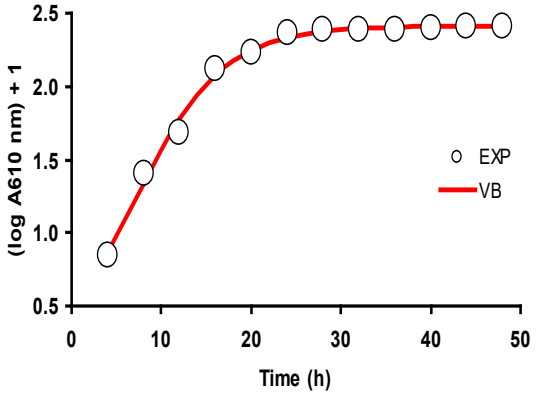

Fig. 7. Growth of *Pseudomonas Putida* (Naun-16) as modelled using the von Bertalanffy model.

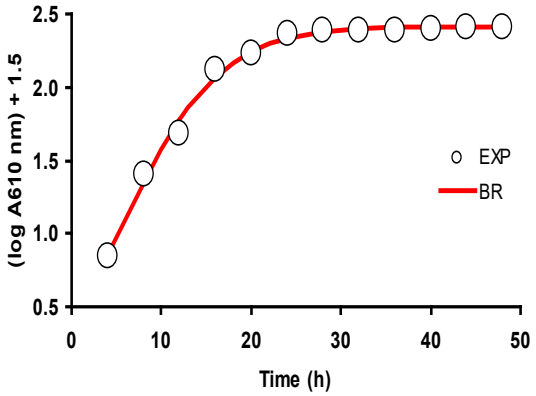

Fig. 8. Growth of *Pseudomonas putida* as modelled using the Baranyi-Roberts model.

Table 2. Statistical tests for the various models utilised in modelling the growth curve of *Pseudomonas Putida* (Naun-16).

Model	*p*	RMSE	R^2	$AdjR^2$	AF	BF	AICc
Huang	4	0.070	0.986	0.978	1.025	1.001	-40.82
Baranyi-Roberts	4	0.043	0.995	0.992	1.014	1.000	-52.18
modified Gompertz	3	0.046	0.993	0.991	1.025	1.000	-57.66
Buchanan-3-phase	3	0.066	0.986	0.981	1.025	1.001	-49.11
modified Richards	4	0.045	0.994	0.991	1.015	1.001	-51.29
modified Schnute	3	0.045	0.994	0.991	1.015	1.001	-51.29
modified Logistics	3	0.042	0.994	0.992	1.015	1.001	-59.57
von Bertalanffy	4	0.049	0.992	0.989	1.016	1.000	-55.99

Note: p is no of parameter

Table 3. Growth coefficients as modelled using the modified Logistic model.

	Y_{max}	μ_{max} (h^{-1})	Lag (H)
Value	2.41	0.12	-3.16
Std Err	0.02	0.01	0.65
Range (95% Confidence)	2.37 to 2.45	0.11 to 0.13	-4.64 to -1.68

The modified logistic model was long introduced [14] and has found applications in modelling bacterial growth [13,25,28,29]. The Huang model was recently introduced [30], but has found applications in modelling bacterial growth in various substrates such as the growth of *Pseudomonas* spp. in pallet-package pork at 10 °C [31], the growth of *Phyllosticta citricarpa* McAlp Van der Aa; the citrus black spot disease [32] and modelling the growth of *Klebsiella pneumoniae* on 2-methylquinoline [33].

Parameters obtained from the fitting exercise were maximum growth rate (μ_{max}), lag time (λ) and maximal growth (Y_{max}). In basic research, these mechanistic models are used and are meant to reach a clear picture of the physical, chemical and biological processes that lead to the growth profile seen. All other things being equal, mechanistic models are more powerful since they tell you about the fundamental procedures driving designs. They are more probable to work properly when concluding beyond the observed conditions. More so, These biologically eloquent coefficients will specially maximum growth rate is useful for secondary modelling exercise using more complex "secondary models" such as Luong, Hans and Levenspiel, Webb, Yano, Aiba and Haldane which will give an insight like the effect of substrate degradation on bacterial growth rate [12,25]. The modelling shows that cyanide is toxic to bacterial growth and also to the environment resulting in a decrease in the maximum growth. The lag period was not severely affected suggesting that probably the cells was able to overcome the toxicity of caffeine at the beginning of growth. However, the growth rate was found to decrease which indicates the cellular growth process is affected by cyanide.

CONCLUSION

In conclusion, The Huang model was the best model in Modelling *Pseudomonas putida* growth curve on cyanide shows that modified Logistics was the best model based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and Akaike Information Criterion with correction (AICc). Parameters obtained from the fitting exercise were maximum growth rate (μ_{max}), lag time (λ), maximal growth (Y_{max}) and minimal growth (Y_o). The use of bacterial growth models to obtained exact growth rate is advantageous for further development of secondary model and this work has revealed the capability of such models.

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