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Modelling the Growth Inhibition Kinetics of *Pseudomonas* sp. strain DrYJ7 on Acrylamide

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ABSTRACT

The bacterium *Pseudomonas* sp. strain DrYJ7 is an efficient acrylamide-degrader and is able to tolerate high concentrations of acrylamide. A primary modelling exercise for the growth of this bacterium on acrylamide yields important specific growth rates which were utilized successfully for secondary modelling exercise which gave Luong as the best model. The statistical analysis and accuracy of the all six kinetic models used indicated that Luong was the best model with small values for RMSE and AICc, uppermost adjusted R^2 values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0). The Luong's constants; maximal growth rate, half-saturation constant for maximal growth, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by μ_{max}, K_s, S_m, and n (± standard error) were 0.66 per day (95% C.I., 0.51 to 0.82), 18.29 mg/L (95% C.I., -17.51to 54.10), 5198 mg/L (95% C.I., 4642 to 5755) and 1.37 (95% C.I., 0.54 to 2.21), respectively. The Luong model predicted the limit, upon which bioremediation of acrylamide will cease.

INTRODUCTION

Environmental issues are increasing rapidly as thousands of various hazardous chemicals are released every day as a result of human activities. Demands are widely pursued for safe and controllable environmentally pollutant alternatives with reduced environmental impact [1]. Acrylamide (CH_2=$CHCONH_2$) is an amide group consisting of three-carbon compound with an α, β-unsaturated olefin bond. This compound is used to make polymers, particularly polyacrylamide, as a commercial conjugated reactive molecule [2–4]. Acrylamide is used as a binding, thickening and flocculating agent worldwide in the industry. [5,6]. Acrylamide is also used to stop soil erosion and in wastewater disposal systems, as pesticide ingredients, cosmetics products, sugar processing. The repeated use of acrylamide and polymers (polyacrylamide) pollute ground and sea [2,3]. Acrylamide is a rising dangerous pollutant. Acrylamide enters the body via ingestion, the skin, lungs and digestive tract [7]. Human reaction to acrylamide is primarily via its exposure to skin impacting the monomer acrylamide and of respiratory dust and vapor. Acrylamide is a recognized mammalian neurotoxicant, carcinogen and terratogen [5]. Acrylamide exerts its toxic effect when it is oxidized to the epoxide glycidamide that catalyzed by an enzymatic reaction involving cytochrome P450 2E1[8]. Previous experiments also shown that acrylamide in animal and plant cells and its oxidized type glycidamide also induced abnormalities. [9]. Given that acrylamide is harmful to human health, it must be eliminated from the atmosphere. Previously, several microorganisms such as *Pseudomonas* sp. [10], *Pseudomonas stutzeri* [11], *Pseudonocardia thermophila* [12], *Bacillus cereus* [13], the fungi *Aspergillus oryzae* [14] and yeast (KCTC 11960BP) [15].

Lately, many statistical models have been used to describe the synthesis of compounds in the natural world which are exposed to microbial communities. Monod is one of the most often used mathematical equations in defining the use of substrates related to growth rate [16]. The restriction of this approach is it is not able to cater for substrate inhibition to the rate. Due to this, other models such as Haldane or other inhibitory models was built on this basis including Aiba, Webb (Edward), Teissier Yano and Koga, Hans-Levenspiel and Luong [17,18]. Hence, the utilization of considerable models available could replace the Haldane in some circumstances and discloses mechanistic process. To date, limited statistical tests were used to accept the best model in modelling the kinetics of xenobiotics biodegradation, and the most commonly used test is the coefficient of determination (R^2) [19,20]. However, by using this coefficient of determination (R^2), the number of parameters used in the model needs to be adjusted [21–23]. This adjustment can be made using an adjusted coefficient of determination (adjR^2), root mean square error RMSE, Corrected Akaike Information Criteria (AICc) and others. In this present study the growth rate on acrylamide was studied using various kinetic models.

MATERIALS AND METHODS

Growth and maintenance of acrylamide-degrading bacterium

Pseudomonas sp. strain DrYJ7 was previously isolated from Antarctica [24] and was maintained in minimal salts medium (MSM). The MSM (pH 7.5) with glucose autoclaved separately is composed of (per liter): 6.8 g of KH_2PO_4 (BDH),10 g of glucose (BDH (British Drug House), Poole, UK), 0.005 g of $FeSO_4 \cdot H_2O$ (BDH), 0.5 g of $MgSO_4 \cdot 7H_2O$ (BDH), various concentrations of acrylamide as the sole nitrogen source with 1 mL of the following trace elements (per liter): 0.003 g of $CoCl_2 \cdot 6H_2O$, 0.01 g of $Cu(CH_3COO)_2.H_2O$ 0.03 g of $ZnCl_2$ (BDH); 0.002 g of $FeCl_2 \cdot 6H_2O$ (JT Baker) and 0.05 g of H_3BO_3 (JT Baker, John Townsend Baker, Phillipsburg, N.J., U.S.A.) . In order to avoid degradation via heating, acrylamide was sterilized by passing through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter. The culture was incubated on a shaking incubator (Certomat R, USA) at 15 °C at 150 rpm for 96 h [24]. Growth was monitored as CFU/mL using appropriate serial dilution of culture periodically sampled from the flask.

Growth kinetics on acrylamide

The bacterial growth kinetics on acrylamide was studied using a batch culture of the bacterium supplemented with acrylamide at concentrations of up to 5000 mg/L. The modified Gompertz model was utilised in the primary inhibition kinetics modelling to obtain the growth parameter maximum specific growth rate or μ_m. The equation is as follows;

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\} \qquad (1)$$

The values obtained from this primary modelling exercise (**published elsewhere**) was then used to model various growth kinetics model as follows;

Table 1. Kinetic models for growth of bacterium on acrylamide.

Author	Degradation Rate	Author
Monod	$\mu_{\max}\frac{S}{K_s+S}$	[16]
Haldane	$\mu_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[25]
Teissier	$\mu_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[26,27]
Aiba	$\mu_{\max}\frac{S}{K_s+S}\exp(-KP)$	[28]
Yano and Koga	$\frac{\mu_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[29]
Luong	$\mu_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[30]

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

Fitting of the data

Nonlinear regression was carried out using the CurveExpert Professional software (Version 1.6), which utilizes the Marquardt algorithm to fit the Gompertz and several inhibition kinetics models (**Table 1**) by nonlinear regression. This algorithm reduces the sums of squares of the residuals.

Statistics of the growth kinetics

Statistical analysis of the growth models' residuals was carried out to select the best model, using approaches such as the corrected Akaike Information Criterion or AICc, adjusted coefficient of determination (R^2), root-mean-square error (RMSE) accuracy factor (AF) and bias factor (BF). Statistical diagnosis tests for normality which are Wilks-Shapiro, Kolmogorov-Smirnov, and D'Agostino-Pearson were performed on the residuals from the Luong model [31].

The RMSE was calculated according to equation 2,

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

where
n number of experimental data
Pd_i predicted values by the model
Ob_i experimental data
p parameters number of the model
In general, the model having the smaller number of parameter results in a smaller RMSE value [32].

The coefficient of determination or R^2 although popular the method does not consider the number of parameters of models in nonlinear regression, and therefore does not readily offer comparative evaluation. To get over this problem, an adjusted R^2 which takes into consideration the quantity of parameter of models is utilized to calculate the quality of nonlinear models based on the formula below;

$$Adjusted\ \left(R^2\right)=1-\frac{RMS}{s_Y^2} \qquad \text{(Eqn. 3)}$$

$$Adjusted\ \left(R^2\right)=1-\frac{\left(1-R^2\right)(n-1)}{(n-p-1)} \qquad \text{(Eqn. 4)}$$

where

$S_y^2$is the total variance of the y-variable and RMS is the Residual Mean Square

The Akaike information criterion (AIC) is established upon information theory. The formula incorporates some variables penalty where the more the variables, the higher the AIC value. In studies where the data is small a corrected version of AIC; the Akaike information requirements (AIC) with correction or AICc is utilised instead [33]. AICc is calculated using the following equation;

$$AICc=2p+n\ln\left(\frac{RSS}{n}\right)+2(p+1)+\frac{2(p+1)(p+2)}{n-p-2} \qquad \text{(Eqn. 5)}$$

Where

n number of data points

p parameter numbers of the model

The Accuracy Factor (AF) and Bias Factor (BF) are another goodness-of-fit exercises for models [34]. The statistics calculates the perfect match between experimental and predicted values. As a rule, a BF value > 1.0 indicates a model which is fail-safe a value < 1.0 indicates a model that is fail-dangerous. On the other hand, the AF is always ≥ 1.0, with precise models giving values nearing to 1.0.

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \qquad \text{(Eqn. 6)}$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{|(Pd_i/Ob_i)|}{n}\right)} \qquad \text{(Eqn. 7)}$$

RESULTS AND DISCUSSION

Growth kinetics

The growth rate of the acrylamide-degrading bacterium on acrylamide as a nitrogen source shows maximal rate at acrylamide concentrations ranging from 100 to 1000 mg/L and also substrate inhibition to the rate with a complete cessation of the growth rate was observed at acrylamide concentration of 5000 mg/L.

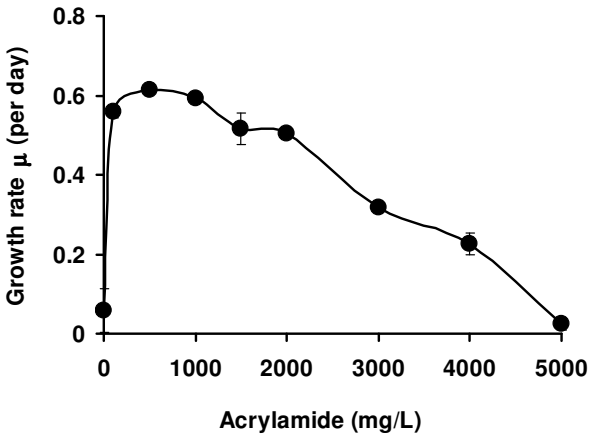

Fig. 1. Growth rate of *Pseudomonas* sp. strain DrYJ7 on various acrylamide concentrations. Error bars indicate mean standard deviation (n=3).

Modelling was carried out using several other kinetic models (**Figs. 2-7**). The statistical analysis and accuracy of the all six kinetic models used indicated that Luong was the best model with small values for RMSE and AICc, uppermost adjusted R^2 values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0) (**Table 2**). The Luong's constants; maximal growth rate, half-saturation constant for maximal growth, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by μ_{max}, K_s, S_m, and n were 0.66 per day (95% C.I., 0.51 to 0.82), 18.29 mg/L (95% C.I., -17.51 to 54.10), 5198 mg/L (95% C.I., 4642 to 5755) and 1.37 (95% C.I., 0.54 to 2.21), respectively. due The restrictions of previous models such Haldane, Andrews Noack, Web, and Yano, alternative models such as Luong, Teissier and Hans-Levenspiel were developed in that certain cases in which growth rate at very high substratum concentration became zero did not justify the use of these models [35].

To date, this is the second time that such a modelling exercise was utilized to model growth kinetics on acrylamide. Modelling the bacterial growth kinetics on toxicants is an essential part of improving successful bioremediation strategies since the obtained consistencies can be used to prepare and consider bioremediation limitations [18]. In a previous study, an acrylamide-degrading yeast also shows the Luong model as the best model with the Luong's constants μ_{max}, K_s, S_m, and n (± standard error) were 0.099±0.017 hr^{-1}, 17.34 ± 5.0 mg/L, 2053.0 ±56.0 mg/L and 0.801±0.202, respectively [36].

After normalization, the specific maximal growth rate on acrylamide of the yeast fares better than the bacterium in this study suggesting a more efficient acrylamide degradation in the yeast. However, the bacterium can tolerate a far higher acrylamide concentration than the yeast judging by the S_m value.

Table 2. Statistical analysis of kinetic models.

Model	p	RMSE	adR2	AICc	BF	AF
Luong	4	0.035	0.973	-27.391	1.027	1.072
Yano	4	0.071	0.875	-14.948	1.188	1.267
Tessier-Edward	3	0.226	-3.574	-6.421	1.088	1.448
Aiba	3	0.260	-0.993	-3.883	0.917	1.921
Haldane	3	0.097	0.748	-21.668	1.231	1.356
Monod	2	0.222	-2.398	-14.571	1.185	1.829
Han and Levenspiel	5	n.a.	n.a.	n.a.	n.a.	n.a.

Note:
p no of parameter
RMSE Root Mean Squared Error
adR^2 Adjusted Coefficient of Determination
AICC Corrected Akaike Information Criterion
BF Bias Factor
AF Accuracy Factor

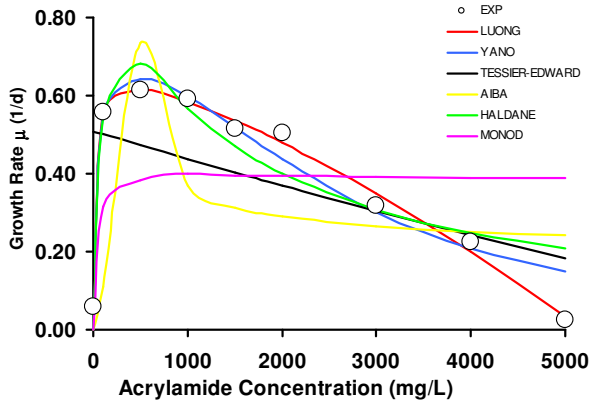

Fig. 3. Curve fitting of the growth rate of *Pseudomonas* sp. strain DrYJ7 on acrylamide using various model.

CONCLUSION

To conclude, the key modelling practice for the growth of this acrylamide by bacterium yields substantial real growth rates which have successfully been used with Luong as the best model in the secondary modelling exercises. The Luong's constants; maximal growth rate, half-saturation constant for maximal growth, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by μ_{max}, K_s, S_m, and n were 0.66 per day (95% C.I., 0.51 to 0.82), 18.29 mg/L (95% C.I., -17.51 to 54.10), 5198 mg/L (95% C.I., 4642 to 5755) and 1.37 (95% C.I., 0.54 to 2.21), respectively. Acrylamide is poisonous and completely inhibits acrylamide degradation and growth on this substrate as according to the Luong model suggesting that to a certain limit, bioremediation might not be successful. To date, a simulation exercise like this has been used to model acrylamide growth kinetics.

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