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# Remodelling the Growth Inhibition Kinetics of *Pseudomonas* sp. Strain DrY Kertih on Acrylamide

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**ABSTRACT**

The bacterium *Pseudomonas* sp. strain Dr Y Kertih is an efficient acrylamide-degrader isolated from hydrocarbon sludge and is able to tolerate high concentrations of acrylamide. Modelling was carried out using several other kinetic models such as Haldane, Andrews Noack, the Web and Yano, Luong, Teissier and Hans-Levenspiel. The statistical analysis and accuracy of the all seven kinetic models used indicated that Aiba was the best model with small values for RMSE and AICc, closest to unity for adjusted $R^2$ values, and Bias Factor and Accuracy Factor nearest to unity. The Aiba's constants; maximal growth rate, half-saturation constant for maximal growth and inhibition constant represented by $\mu_{max}$, $K_s$ and $K_i$, were 0.221 per h (95% C.I., 0.140 to 0.301), 128.9 mg/L (95% C.I., 40.4 to 217.4) and 633.8 mg/L (95% C.I., 458.6 to 808.9), respectively. The true maximal reduction rate, which occurred when the slope of the curve is zero occurs at 229 mg/L acrylamide concentration and a corresponding value of 0.099 $hr^{-1}$ (95% C.I., 0.063 to 0.135). The modelling of toxicant bacterial growth kinetics is an integral part of improving effective bioremediation strategies as the consistencies obtained can be used to plan and strategize bioremediation constraints. To date, based on the specific maximal growth rate on acrylamide for this bacterium, it indicates it is the most efficient acrylamide-degrading strain.

## INTRODUCTION

When thousands of different toxic chemicals are released from human activity every day, the pollution crisis is growing exponentially. Global needs for environmentally sustainable and healthy solutions with a lower environmental impact are increasingly being voiced at the highest level [1]. Acrylamide ($CH_2$=$CHCONH_2$) is a compound used to make polymers, particularly polyacrylamide, as a commercial conjugated reactive molecule and is an amide group consisting of three-carbon compound with an $\alpha,\beta$-unsaturated olefin bond [2–4]. Acrylamide is primarily used to stop soil erosion and in wastewater disposal systems, as pesticide ingredients, cosmetics products, sugar processing, binding, thickening and flocculating agent worldwide in various industries [5,6].

Acrylamide is a rising dangerous pollutant with repeated use of acrylamide and polymers (polyacrylamide) pollutes the ground and sea [2,3]. Human reaction to acrylamide is primarily via its exposure to skin, via ingestion, lungs and the digestive tract [7]. Acrylamide exerts its toxic effect when it is oxidized to the epoxide glycidamide that catalyzed by an enzymatic reaction involving cytochrome P450 2E1 [8]. It is a recognized mammalian neurotoxicant, carcinogen and terratogen [5].

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, Microorganisms that have been reported as capable of utilizing acrylamide include the yeast *Rhodotorula* sp.[10], bacteria *Enterobacter aerogenes* [11], *Pseudomonas* sp. [12] *Burkholderia* sp. [13], *Bacillus cereus* [12] an Antarctic bacterium [12] and the fungi *Aspergillus oryzae* [14]. *Pseudomonas* is a special strain which can degrade acrylamide and also various toxicants such as reducing molybdate to molybdenum blue [12,15], growth on SDS and degrade diesel [16,17]. *Pseudomonas* sp. has also been suggested as heavy metal remover [18–20]. Examples of *Pseudomonas* sp. that can degrade acrylamide are *Pseudomonas stutzeri* [21] and *Pseudomonas chlororaphis* [22].

Previously, we have isolated an acrylamide-degrading bacterium from hydrocarbon sludge and describes its growth kinetics on acrylamide using the popular Haldane model without resorting to other available substrate-inhibiting kinetic models such as Luong, Teissier-Edward, Yano, Aiba and Hans-Levenspiel. In this study we reevaluate the modelling exercise by incorporating more model as mentioned above.

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 [23]. 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 [24,25]. 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 ($R^2$) [26,27]. However, by using this coefficient of determination ($R^2$), the number of parameters used in the model needs to be adjusted [28–30]. This adjustment can be made using an adjusted coefficient of determination (adj$R^2$), 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 Dr Y Kertih was previously isolated from Antarctica [31] and was maintained in minimal salts medium (MSM). In order to avoid degradation via heating, acrylamide was sterilized by passing through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter. 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.). The culture was incubated on a shaking incubator (Certomat R, USA) at 27 °C at 150 rpm for 96 h [31]. 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 (**published elsewhere**) to obtain the growth parameter maximum specific growth rate or $\mu_m$. The equation is as follows;

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

The values obtained from this primary modelling exercise 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 | $\mu_{\max}\frac{S}{K_S+S}$ | [23] |
| Haldane | $\mu_{\max}\frac{S}{S+K_S+\frac{S^2}{K_i}}$ | [32] |
| Teissier | $\mu_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_S}\right)\right)$ | [33,34] |
| Aiba | $\mu_{\max}\frac{S}{K_S+S}\exp(-KP)$ | [35] |
| Yano and Koga | $\frac{\mu_{\max}S}{S+K_S+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$ | [36] |
| Luong | $\mu_{\max}\frac{S}{S+K_S}\left[1-\left(\frac{S}{S_m}\right)^n\right]$ | [37] |

Note:
$\mu_{max}$ 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

To select the best model, statistical analysis of the growth models' residuals was carried out , using error function analyses such as corrected Akaike Information Criterion or AICc (n<40), adjusted coefficient of determination ($R^2$), root-mean-square error (RMSE), accuracy factor (AF) and bias factor (BF).

The RMSE was calculated according to equation 2,

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

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 [38]. 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 $R^2$ (**Eqns. 3** and **4)** which takes into consideration the quantity of parameter of models is utilized to calculate the quality of nonlinear models based on the formula below;

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

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

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 [39]. AICc is calculated using the following equation (**Eqn. 5)**;

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

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 (Ross and McMeekin, 1994). 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 (**Eqns. 6** and **7)**.

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

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

## 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 200 to 400 mg/L and also substrate inhibition to the rate at acrylamide concentrations higher than this range [31].

Modelling was carried out using several other kinetic models (**Fig. 1**). The statistical analysis and accuracy of the all six kinetic models used indicated that Aiba 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 Aiba's constants; maximal growth rate, half-saturation constant for maximal growth and inhibition constant represented by $\mu_{max}$, $K_s$ and $K_i$, were 0.221 per h (95% C.I., 0.140 to 0.301), 128.9 mg/L (95% C.I., 40.4 to 217.4) and 633.8 mg/L (95% C.I., 458.6 to 808.9), respectively. The true maximal reduction rate, which occurred when the slope of the curve is zero [40] occurs at 229 mg/L acrylamide concentration and a corresponding value of 0.099 $hr^{-1}$ (95% C.I., 0.063 to 0.135). In a previous modelling exercise, the Haldane model was found to be the best.

In some cases, which such as growing at very high substrate concentrations, there appears to be restrictions to earlier models such as Haldane, Andrews Noack, the Web and Yano resulting in the development of alternative models such as Luong, Teissier and Hans-Levenspiel [41]. To date, this is the first time that the Aiba's model was found to be the best for studying bacterial substrate inhibition when grown on acrylamide.

The modelling of toxicant bacterial growth kinetics is an integral part of improving effective bioremediation strategies as the consistencies obtained can be used to plan and strategize bioremediation constraints [25]. In a previous study, an acrylamide-degrading yeast also shows the Luong model as the best model with the Luong's constants $\mu_{max}$, $K_s$, $S_m$, 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 [42]. In comparison, the specific maximal growth rate on acrylamide for this bacterium indicates it has a higher efficiency than the acrylamide rate of growth on yeast.

The Aiba model has been used in many biological processes with good success. Examples include ammonium and nitrate oxidation rate in a suspended biomass system (SBS) in the nitratation processes in an immobilised biomass system [43] and the bioconversion of wastewater by the photosynthetic bacteria [44]. The Aiba model was also used in modelling the hydrogen production by cyanobacteria [45] and the rate of quinoline biodegradation and mineralization by an internal loop photo-biodegradation reactor [46].

Other examples include in the glyphosate degradation rate by *Bacillus subtilis* [47], the degradation rate of tributyl tin by *Klebsiella* sp. [48], nonyphenol biodegradation [49], cresol biodegradation [50] and crude glycerol fermentation to dihydroxyacetone by immobilized *G. oxydans* cells [51]. It is also the best model in modelling manganese oxidation rate by *Streptomyces violarus* strain SBP1 [52] and the inhibitory effect of ethanol on ethanol fermentation by *Kluyveromyces* sp. IIPE453 [53].

**Table 2.** Statistical analysis of kinetic models.

| Model | p | RMSE | adR$^2$ | AICc | BF | AF |
|---|---|---|---|---|---|---|
| Luong | 4 | 0.003 | 0.987 | -71.552 | 1.000 | 1.027 |
| Yano | 4 | 0.003 | 0.986 | -70.709 | 1.002 | 1.025 |
| Tessier-Edward | 3 | 0.004 | 0.980 | -79.576 | 1.001 | 1.033 |
| Aiba | 3 | 0.003 | 0.986 | -82.624 | 1.001 | 1.028 |
| Haldane | 3 | 0.005 | 0.969 | -75.839 | 1.005 | 1.046 |
| Monod | 2 | 0.018 | 0.437 | -60.143 | 0.963 | 1.189 |
| Han and Levenspiel | 5 | 0.003 | 0.989 | -49.236 | 1.000 | 1.189 |

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

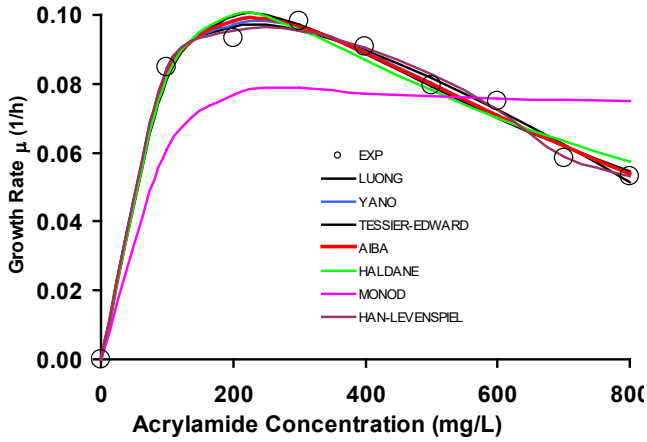


**Fig. 3.** Curve fitting of the growth rate of *Pseudomonas* sp. strain Dr Y Kertih on acrylamide using various model.

## 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 $\mu_{max}$, $K_s$, $S_m$, 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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