Growth Inhibition Kinetics of a Pseudomonas Diesel-degrading Strain from Antarctica

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INTRODUCTION

Oil spills which cause hydrocarbon pollutants are amongst the most reported pollution worldwide. Even pristine areas especially in the polar regions have been affected \cite{1}. Their negative impacts on various organisms \cite{2–4} have been documented and have prompted many researchers to search for solutions to remove these pollutants from the environment. The removal of pollutants particularly hydrocarbons are very challenging as temperature, climate and geographical aspects play major role and need to be carefully considered when finding the most effective bioremediation solution. One of the largest sources of hydrocarbon contaminant in the Polar Regions is oil spills from shipping accidents. Several of the most notable examples are the Exxon Valdez, Bahia Paraiso and Nella Dan accidents. It has been observed that the soils and sediments contaminated with hydrocarbon are teemed with hydrocarbon-degrading microorganisms in the form of bioremediation to prepare for future disasters. A previously isolated diesel-degrading Pseudomonas sp. strain DRYJ3 has shown effectiveness as a bioremediation tool. Its growth is however strongly inhibited as the diesel concentrations was increased. In this study the inhibitory effect of diesel on the growth rate of this bacterium is modelled according to the Luong, Aiba, Haldane, Hans-Levenspiel, Yano, Teissier and Monod models. Statistical evaluations indicated that the most suitable kinetic model to fit the growth rate on diesel was Luong’s model. The Luong’s constants; maximal growth rate, half saturation constant for maximal growth, maximum substrate concentration that growth ceases, and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by $\mu_{\text{max}}$, $K_s$, $S_m$, and $n$ were $0.406 \text{ hr}^{-1}$ (95% CI, 0.269 to 0.881), $0.194 \text{ (%v/v)}$ (95% CI, 0.2877 to 0.390), $4.025 \text{ (%v/v)}$ (95% CI, 3.820 to 4.229) and $0.378$ (95% CI, 0.122 to 0.877), respectively. The Luong model predicted $S_m$ value was close to the value of which no growth was observed experimentally suggesting the appropriateness of the model in adhering to observed values.

KEYWORDS

Pseudomonas sp.
Antarctica
diesel-degrading
growth kinetics
Luong

ABSTRACT

Antarctica is one of the largest southernmost continent and most pristine wilderness areas left on earth. Over decades, human activities in this area have resulted in the accumulated pollution of hydrocarbon in the Antarctica mainly due to transportation and logistics activities. The sinking of the supply ships \textit{Nella Dan} and \textit{Bahia Paraiso} have resulted in diesel spillage that warrant the utilization and research on diesel-degrading microorganisms in the form of bioremediation to prepare for future disasters. A previously isolated diesel-degrading \textit{Pseudomonas} sp. strain DRYJ3 has shown effectiveness as a bioremediation tool. Its growth is however strongly inhibited as the diesel concentrations was increased. In this study the inhibitory effect of diesel on the growth rate of this bacterium is modelled according to the Luong, Aiba, Haldane, Hans-Levenspiel, Yano, Teissier and Monod models. Statistical evaluations indicated that the most suitable kinetic model to fit the growth rate on diesel was Luong’s model. The Luong’s constants; maximal growth rate, half saturation constant for maximal growth, maximum substrate concentration that growth ceases, and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by $\mu_{\text{max}}$, $K_s$, $S_m$, and $n$ were $0.406 \text{ hr}^{-1}$ (95% CI, 0.269 to 0.881), $0.194 \text{ (%v/v)}$ (95% CI, 0.2877 to 0.390), $4.025 \text{ (%v/v)}$ (95% CI, 3.820 to 4.229) and $0.378$ (95% CI, 0.122 to 0.877), respectively. The Luong model predicted $S_m$ value was close to the value of which no growth was observed experimentally suggesting the appropriateness of the model in adhering to observed values.
Antarctica. The relation between the specific growth rate ($\mu$) of a population of microorganisms and the substrate concentration ($S$) is a valuable tool in biotechnology.

The Monod equation has been widely used to describe growth-linked substrate utilization rate [10,11]. However, when a substrate such as diesel exhibits inhibition towards its own biodegradation, the original Monod model could not be used. In this case, its derivatives that have new constants that provided corrections for substrate have been devised instead. A variety of microbial growth of for this work biodegradation kinetic models are available. The generalization of the use of the Haldane model in literature to model substrate inhibition to growth or degradation rate is numerous literatures. This is despite the fact, that for a single substrate-inhibiting compound such as phenol, several other models have been demonstrated to be more accurate. For instance, aside from the predominantly reported Haldane model [12], several other different models have been found to be optimal such as Luong [13,14] and Edward [15]. Hence, the use of extensive models available could replace the Haldane in some circumstances. Without actually fitting these other models to the available growth or degradation rate data and proper statistical evaluation, the exclusive use of the Haldane model should not be used liberally.

Previously, a diesel-degrading bacterium has been isolated from Antarctic soils and the kinetics of the growth of the bacterium has not been determined using various inhibitory growth kinetics models available [16] as shown in Table 1. This study is carried out to evaluate the use of such models in determining the effect of diesel on the growth rate of this bacterium.

<table>
<thead>
<tr>
<th>Author</th>
<th>Degradation Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monod</td>
<td>$q_{max} \frac{S}{K_s + S}$</td>
</tr>
<tr>
<td>Haldane</td>
<td>$q_{max} \frac{S}{S + K_s}$</td>
</tr>
<tr>
<td>Teissier</td>
<td>$q_{max} \left(1 - \exp \left(-\frac{S}{K_s} \right) \exp \left(-\frac{S}{K_s} \right) \right)$</td>
</tr>
<tr>
<td>Aiba</td>
<td>$q_{max} \frac{S}{K_s + S} \exp(-KP)$</td>
</tr>
<tr>
<td>Yano and Koga</td>
<td>$q_{max} \frac{S}{S + K_s \left(\frac{S}{K_s} \right)^m \left(1 + \frac{S}{K_s} \right)^n}$</td>
</tr>
<tr>
<td>Han and Levenspiel</td>
<td>$q_{max} \left[1 - \left(\frac{S}{S_p} \right)^n \right] \left[1 + \left(\frac{S}{S_p} \right)^m \right]$</td>
</tr>
<tr>
<td>Luong</td>
<td>$q_{max} \frac{S}{S + K_s} \left[1 + \left(\frac{S}{S_p} \right)^n \right]$</td>
</tr>
</tbody>
</table>

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

### MATERIALS AND METHODS

**Growth and Maintenance of Pseudomonas sp. strain DRYJ3**

Antarctica isolated *Pseudomonas* sp. strain DRYJ3 from [16] was grown in a 100 ml media consisted of a modified basalt media (BSM) at pH 7.0. The modified BSM was composed of (per liter of distilled water): KH₂PO₄, 1.360 g; Na₂HPO₄, 1.388 g; KNO₃, 0.5 g; MgSO₄, 0.01 g; CaCl₂, 0.01 g; (NH₄)₂SO₄, 7.7 g; and 100 ml of a mineral solution containing 0.01 g of ZnSO₄·7H₂O, MnCl₂·4H₂O, H₂BO₃, CoCl₂·6H₂O, FeSO₄·2H₂O, CuCl₂·H₂O, NaMoO₄·2H₂O. The flasks were incubated at 10±0.5 °C and 150 rpm (YIH DER, Taiwan) [16]. Diesel was the only carbon source supplied for bacterial growth and acclimatization. The growth of the bacterium at various concentrations of diesel was monitored by the plate count method and the specific growth rate at each diesel concentrations was obtained via nonlinear regression using the modified Gompertz model (results published elsewhere).

**Fitting of the data**

The nonlinear equations were fitted to the growth data by nonlinear regression with a Marquardt algorithm that minimizes sums of square of residuals using CurveExpert Professional software (Version 1.6). This is a search method to minimize the sum of the squares of the differences between the predicted and measured values.

**Statistical analysis**

To decide whether there is a statistically substantial difference between models with different number of parameters, in terms of the quality of fit to the same experimental 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).

The RMSE was calculated according to Eq. (1), where $P_i$ are the values predicted by the model and $O_i$ 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 [24] [9].

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (P_i - O_i)^2}{n - p}} \quad \text{(Equation 1)}$$

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 $S_y^2$ is the total variance of the y-variable (Eqs. 2 and 3) [9].

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

$$\text{Adjusted } R^2 = 1 - \frac{(1 - R^2)(n-1)}{(n-p-1)} \quad \text{(Equation 3)}$$
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 [25]. For an output of a set of predicted model, the most preferred model would be the model showing 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 equation incorporates number of parameters penalty, the more the parameters, the less preferred the output or the higher the AIC value. Hence, AIC not merely rewards goodness of fit, but in addition does not encourage using more complicated model (overfitting) for fitting experimental data. Since the data in this work is small compared to the number of parameters used a corrected version of AIC, the Akaike information criterion (AIC) with correction or AICc is used instead. The AICc is calculated for each data set for each model according to the following equation (Eqn. 4) [9]:

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

(Equation 4)

Where \(n\) is the number of data points and \(p\) is the number of parameters of the model. The method takes into account the change in goodness-of-fit and the difference in number of parameters between two models. For each data set, the model with the smallest AICc value is highly likely correct [24].

Accuracy Factor (AF) and Bias Factor (BF) to test for the goodness-of-fit of the models as suggested by Ross [26] were also used (Eqns. 5 and 6). Halmi et al., [27] have also adopted the same model. The Bias Factor equal to 1 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 > 1 indicates a fail-safe model. The Accuracy Factor is always ≥ 1, and higher AF values indicate less precise prediction.

$$\text{Bias factor} = 10^{\left(\frac{\sum_{i=1}^{n} \log\left|\frac{P_{i}}{O_{i}}\right|}{n}\right)}$$

(Equation 5)

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

(Equation 6)

RESULTS AND DISCUSSION

Seven different growth models (Table 1) were used in this study to match the experimental data as studied by Halmi et al. (2016). The resultant fitting shows visually acceptable fitting (Fig. 1) with the exception of the Monod model. The drawback of the Monod model is that the distinctive, regulatory complex, variation response to environmental factors, and capacity of microbes to generate various products and by-products in inherent metabolism is disregarded. Statistical analysis (Table 2) showed the Luong model as the best model with best results for all error functions analysed.

![Fig 1. The growth of curves of Pseudomonas sp. strain DRYJ3 on various concentrations of diesel. The coefficient of variation for all data was less than 10% for all data and error bars were omitted for clarity.](image)

<table>
<thead>
<tr>
<th>Model</th>
<th>(p)</th>
<th>RMSE</th>
<th>(adjR^2)</th>
<th>AICc</th>
<th>AF</th>
<th>BF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luong</td>
<td>4</td>
<td>0.003</td>
<td>1.00</td>
<td>-69.96</td>
<td>0.99</td>
<td>1.03</td>
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<tr>
<td>Alba</td>
<td>3</td>
<td>0.010</td>
<td>0.97</td>
<td>-62.66</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Haldane</td>
<td>3</td>
<td>0.021</td>
<td>0.81</td>
<td>-48.82</td>
<td>1.03</td>
<td>1.14</td>
</tr>
<tr>
<td>Han and</td>
<td>4</td>
<td>0.028</td>
<td>0.66</td>
<td>-7.64</td>
<td>1.02</td>
<td>1.14</td>
</tr>
<tr>
<td>Yano</td>
<td>4</td>
<td>0.014</td>
<td>0.93</td>
<td>-44.76</td>
<td>1.01</td>
<td>1.06</td>
</tr>
<tr>
<td>Teissier</td>
<td>4</td>
<td>0.019</td>
<td>0.89</td>
<td>-38.76</td>
<td>0.82</td>
<td>1.16</td>
</tr>
<tr>
<td>Monod</td>
<td>2</td>
<td>0.069</td>
<td>-7.60</td>
<td>-35.58</td>
<td>0.82</td>
<td>1.68</td>
</tr>
</tbody>
</table>

Note:
- \(p\) no of parameters
- RMSE Root Mean Square Error
- \(adjR^2\) Adjusted Coefficient of determination
- BF Bias factor
- AF Accuracy factor
The Luong’s constants; maximal growth rate, half saturation constant for maximal growth, maximum substrate concentration that growth ceases, and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by $\mu_{max}$, $K_s$, and $n$ were 0.406 hr$^{-1}$ (95% CI, 0.269 to 0.881), 0.194 (95% CI, 0.2877 to 0.390), 4.025 (95% CI, 3.820 to 4.229) and 0.378 (95% CI, 0.122 to 0.877), respectively. The Luong model predicted $S_{max}$ value was close to the value of which zero growth was examined signifying the relevance of the model in respecting to obtained values.

Models such as Luong, Teissier and Hans-Levenspiel were developed due to the limitations of previous models such Haldane, Andrews and Noack, Web, and Yano in that these models unable to explain certain rare situations where growth rate became zero at very high substrate concentration [28]. In certain cases, at high substrate concentrations, microbial growth rate is inhibited by the substrate itself due to repressive and toxic effects. To date, the majority of the Luong model reported for xenobiotics-degrading bacteria centred on works on phenol-degrading microbial works [13,27,29] and molybdenum-reducing bacterium [30,31] and no work has been reported for degrading microbial works [13,27,29] and molybdenum -xenobiotics-degrading bacteria centred on works on phenol-degrading microbial works. To date, the majority of the Luong model reported for biodegradation due to the limitations of previous models such as Haldane, Andrews and Noack, Web, and Yano in that these models unable to explain certain rare situations where growth rate became zero at very high substrate concentration [28].

In this study, a complete cessation of maximum growth at a very high diesel concentration to the growth rate of the Antarctic bacterium Pseudomonas sp. strain DRYJ3 was observed and the use of various kinetics model in conjunction with a comprehensive statistical treatise of the model suggest that the Luong model was the best in fitting the growth rate at various diesel concentrations as opposed to the popular Haldane model. The Luong model allows for the modelling of the maximum concentration of substrate that results in the complete cessation of growth rate.

**CONCLUSION**

In this study, a complete cessation of maximum growth at a very high diesel concentration to the growth rate of the Antarctic bacterium Pseudomonas sp. strain DRYJ3 was observed and the use of various kinetics model in conjunction with a comprehensive statistical treatise of the model suggest that the Luong model was the best in fitting the growth rate at various diesel concentrations as opposed to the popular Haldane model. The Luong model allows for the modelling of the maximum concentration of substrate that results in the complete cessation of growth rate.

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The Luong equation can then be replaced with the calculated model parameters (Eqn. 7). as follows;

$$
\mu = 0.099 \frac{S}{S + 0.194} \left(1 - \frac{S}{4.025}\right)^{-2.787}
$$

**REFERENCE**

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