BIOREMEDIATION SCIENCE AND TECHNOLOGY RESEARCH

Website: https://journal.hibiscuspublisher.com/index.php/BSTR

BSTR VOL 7 NO 1 2019

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

Farrah Aini Dahalan[1]* and Nor Ayshah Alia Ali Hassan[2]

[1]School of Environmental Engineering, Universiti Malaysia Perlis, Kompleks Pusat Pengajian Jejawi 3,
02600 Arau, Perlis, Malaysia.
[2]Soil, Water, and Fertilizer Research Centre, Malaysian Agricultural Research and Development Institute, Persiaran MARDI-UPM,
43400 Serdang, Selangor.

*Corresponding author:
Dr. Farrah Aini Dahalan,
School of Environmental Engineering,
Universiti Malaysia Perlis,
Kompleks Pusat Pengajian Jejawi 3,
02600 Arau,
Perlis,
Malaysia.
Email: farrahaini@unimap.edu.my

HISTORY

Received: 11th May 2019
Received in revised form: 7th of June 2019
Accepted: 14th of June 2019

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 *Nella Dan* and *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 *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 μ_{max}, K_s, S_m, and n were 0.406 hr^{-1} (95% CI, 0.269 to 0.881), 0.194 (%v/v) (95% CI, 0.2877 to 0.390), 4.025 (%v/v) (95% CI, 3.820 to 4.229) and 0.378 (95% CI, 0.122 to 0.877) 0.099, 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.

INTRODUCTION

Oil spills which causes hydrocarbon pollutants are amongst the most reported pollution worldwide. Even pristine areas especially in the polar regions have been affected [1]. Their negative impacts on various organisms [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 microorganism [5]. An enhanced number of hydrocarbon degraders were reported in hydrocarbon contaminated soils from Scott Base, Marble Point and Wright Valley in Antarctica and in oil-polluted Antarctic seawater [6–8]. Ever since Antarctica is distinct as research base, numerous diesel-degrading bacteria from Antarctica have been isolated for bioremediation study. At this juncture despite these reports, the effect of diesel on the growth rate of diesel-degrading Antarctic bacteria has never been reported. This study adapts the study conducted previously Halmi *et al.* (2016) on modelling the kinetics growth of hexavalent molybdenum (Mo^{6+}) reduction by the Serratia sp. strain MIE2 to study the growth inhibition kinetics of a *Pseudomonas* diesel-degrading strain from

Antarctica. The relation between the specific growth rate (μ) 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 1. Various mathematical models developed for degradation kinetics involving substrate inhibition.

Author	Degradation Rate	Author
Monod	$q_{\max}\dfrac{S}{K_s+S}$	[17]
Haldane	$q_{\max}\dfrac{S}{S+K_s+\dfrac{S^2}{K_i}}$	[18]
Teissier	$q_{\max}\left(1-\exp\left(-\dfrac{S}{K_i}\right)-\exp\left(\dfrac{S}{K_s}\right)\right)$	[19]
Aiba	$q_{\max}\dfrac{S}{K_s+S}\exp(-KP)$	[20]
Yano and Koga	$\dfrac{q_{\max}S}{S+K_s+\left(\dfrac{S^2}{K_1}\right)\left(1+\dfrac{S}{K}\right)}$	[21]
Han and Levenspiel	$q_{\max}\left[1-\left(\dfrac{S}{S_m}\right)\right]^n\left[\dfrac{S}{S+K_s\left(1-\dfrac{S}{S_m}\right)^m}\right]$	[22]
Luong	$q_{\max}\dfrac{S}{S+K_s}\left[1-\left(\dfrac{S}{S_m}\right)^n\right]$	[23]

Note:

q_{max}	maximal degradation rate
K_s	half saturation constant for maximal degradation
S_m	maximal concentration of substrate tolerated
m, n, K	curve parameters
S	substrate concentration
P	product concentration

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 salt media (BSM) at pH 7.0. The modified BSM was composed of (per liter of distilled water): KH_2PO_4, 1.360 g; Na_2HPO_4, 1.388 g; KNO_3, 0.5 g; $MgSO_4$, 0.01 g; $CaCl_2$, 0.01 g; $(NH_4)_2SO_4$, 7.7 g; and 100 ml of a mineral solution containing 0.01 g of $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, H_3BO_4, $CoCl_2.6H_2O$, $Fe_2SO_4.2H_2O$, $CuCl_2.2H_2O$, $NaMoO_4.2H_2O$. 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 *Pd* are the values predicted by the model and *Ob* 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].

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

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 (**Eqns. 2 and 3**) [9].

$$Adjusted\left(R^2\right)=1-\frac{RMS}{s_Y^2} \qquad \textbf{(Equation 2)}$$

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

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];

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

$$\text{Bias factor}=10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)}$$ (**Equation** 5)

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

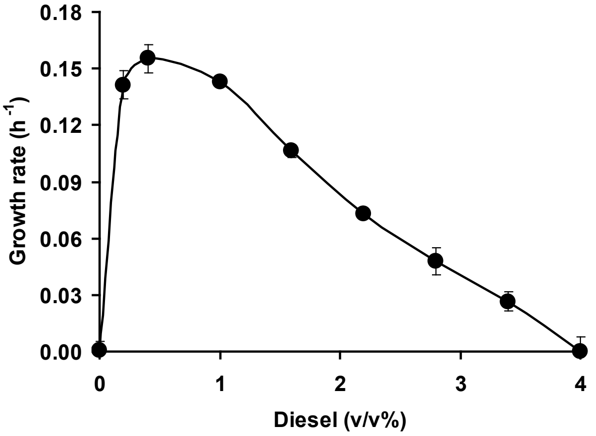

Fig 1. The growth of curves of *Pseudomonas* sp. strain DRYJ3 on various concentrations of diesel. The coefficient of variation for all data was less than 10% for all data and error bars were omitted for clarity.

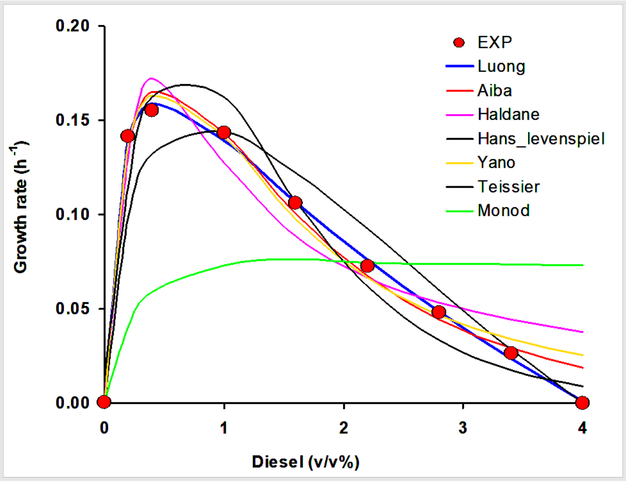

Fig 2. The growth of curve of *Pseudomonas* sp. strain DRYJ3 at 0.1% (v/v) diesel fitted by various growth models available in the literature.

Table 2. Statistical analysis of the various fitting models.

Model	p	RMSE	adR^2	AICc	AF	BF
Luong	4	0.003	1.00	-69.96	0.99	1.03
Aiba	3	0.010	0.97	-62.66	0.99	0.99
Haldane	3	0.021	0.81	-48.82	1.03	1.14
Han and	4	0.028	0.66	-7.64	1.02	1.14
Yano	4	0.014	0.93	-44.76	1.01	1.06
Teissier	4	0.019	0.89	-38.76	0.82	1.16
Monod	2	0.069	-7.60	-35.58	0.82	1.68

Note:

p	no of paramaters
RMSE	Root Mean Square Error
Ra^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 μ_{max}, K_s, S_m, and n were 0.406 hr^{-1} (95% CI, 0.269 to 0.881), 0.194 (%v/v) (95% CI, 0.2877 to 0.390), 4.025 (%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 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 the use of this model in modelling the effect of diesel on the growth rate of bacterium especially Antarctic bacterium to the best of our knowledge.

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

$$\mu = 0.099\frac{S}{S+0.194}\left[1-\left(\frac{S}{4.025}\right)^{0.378}\right] \qquad \text{(\textbf{Equation} 7)}$$

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.

ACKNOWLEDGEMENT

This work was supported by the research grants from The Academy of Science Malaysia (ASM) and The Ministry of Science, Technology and Innovation (MOSTI). We thank Prof Dr. Mac Cormack, Dr. Lucas Ruberto, Dr. Susana Claudia Vázquez and Dr. Edgardo Hernandez from The Argentinean Institute of Antarctica (IAA) for providing site supports, soil samples and sample preparations during the expedition to Antarctica.

REFERENCES

1. Ruberto LAM, Vazquez S, Lobalbo A, Mac Cormack WP. Psychrotolerant hydrocarbon-degrading *Rhodococcus* strains isolated from polluted Antarctic soils. Antarc Sci. 2005;17(1):47–56.
2. Atlas RM, Cerniglia CE. Bioremediation of petroleum pollutants. BioScience. 1995;45(5):332–8.
3. Ku Ahamad KE, Halmi MIE, Shukor MY, Wasoh MH, Abdul Rachman AR, Sabullah MK, et al. Characterization of a diesel-degrading strain isolated from a local hydrocarbon-contaminated site. J Environ Biormed Toxicol. 2013;1(1):1–8.
4. Al-Baldawi IA, Abdullah SRS, Anuar N, Suja F, Mushrifah I. Phytodegradation of total petroleum hydrocarbon (TPH) in diesel-contaminated water using *Scirpus grossus*. Ecol Eng. 2015;74:463–73.
5. Dahalan FA, Yunus I, Johari WLW, Shukor MY, Halmi MIE, Shamaan NA, et al. Growth kinetics of a diesel-degrading bacterial strain from petroleum-contaminated soil. J Environ Biol. 2014;35(2):399–406.
6. Aislabie J, Bej AK, Hurst H, Rothenburger S, Atlas RM. Microbial degradation of quinoline and methylquinolines. Appl Environ Microbiol. 1990;56(2):345–51.
7. Aislabie J, Fraser R, Duncan S, Farrell RL. Effects of oil spills on microbial heterotrophs in Antarctic soils. Polar Biol. 2001;24(5):308–13.
8. Delille D, Bassères A, Dessommes A. Effectiveness of bioremediation for oil-polluted Antarctic seawater. Polar Biol. 1998;19(4):237–41.
9. Halmi MIE, Abdullah SRS, Johari WLW, Ali MSM, Shaharuddin NA, Khalid A, et al. Modelling the kinetics of hexavalent molybdenum (Mo6+) reduction by the Serratia sp. strain MIE2 in batch culture. Rendiconti Lincei. 2016 Dec 1;27(4):653–63.
10. Kiviharju K, Salonen K, Leisola M, Eerikäinen T. Modeling and simulation of Streptomyces peucetius var. caesius N47 cultivation and ε-rhodomycinone production with kinetic equations and neural networks. J Biotechnol. 2006;126(3):365–73.
11. Shokrollahzadeh S, Bonakdarpour B, Vahabzadeh F, Sanati M. Growth kinetics and Pho84 phosphate transporter activity of Saccharomyces cerevisiae under phosphate-limited conditions. J Ind Microbiol Biotechnol. 2007;34(1):17–25.
12. Arif NM, Ahmad SA, Syed MA, Shukor MY. Isolation and characterization of a phenol-degrading *Rhodococcus* sp. strain AQ5NOL 2 KCTC 11961BP. J Basic Microbiol. 2013;53(1):9–19.
13. Hamitouche A-E, Bendjama Z, Amrane A, Kaouah F, Hamane D. Relevance of the Luong model to describe the biodegradation of phenol by mixed culture in a batch reactor. Ann Microbiol. 2012;62(2):581–6.
14. Nickzad A, Mogharei A, Monazzami A, Jamshidian H, Vahabzadeh F. Biodegradation of phenol by Ralstonia eutropha in a Kissiris-immobilized cell bioreactor. Water Environ Res. 2012;84(8):626–34.
15. Saravanan P, Pakshirajan K, Saha P. Batch growth kinetics of an indigenous mixed microbial culture utilizing m-cresol as the sole carbon source. J Haz Mat. 2009;162(1):476–81.
16. Shukor MY, Hassan NAA, Jusoh AZ, Perumal N, Shamaan NA, MacCormack WP, et al. Isolation and characterization of a *Pseudomonas* diesel-degrading strain from Antarctica. J Environ Biol. 2009;30(1):1–6.
17. Monod J. The Growth of Bacterial Cultures. Ann Rev Microbiol. 1949;3(1):371–94.
18. Boon B, Laudelout H. Kinetics of nitrite oxidation by Nitrobacter winogradskyi. Biochem J. 1962;85:440–7.
19. Teissier G. Growth of bacterial populations and the available substrate concentration. Rev Sci Inst. 1942;3208:209–14.
20. Aiba S, Shoda M, Nagatani M. Kinetics of product inhibition in alcohol fermentation. Biotechnol Bioeng. 1968 Nov 1;10(6):845–64.
21. Yano T, Koga S. Dynamic behavior of the chemostat subject to substrate inhibition. Biotechnol Bioeng. 1969 Mar 1;11(2):139–53.
22. Han K, Levenspiel O. Extended Monod kinetics for substrate, product, and cell inhibition. Biotechnol Bioeng. 1988;32(4):430–7.
23. Luong JHT. Generalization of monod kinetics for analysis of growth data with substrate inhibition. Biotechnol Bioeng. 1987;29(2):242–8.
24. Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J, 1987;1(5):365–74.
25. Akaike H. New look at the statistical model identification. IEEE Trans Automat Contrl. 1974;AC-19(6):716–23.
26. Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994;23(3–4):241–64.

27. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modelling of the degradation kinetics of *Bacillus cereus* grown on phenol. J Environ Biormed Toxicol.. 2014;2(1):1–5.
28. Saravanan P, Pakshirajan K, Saha P. Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor. Bioresour Technol. 2008;99(1):205–9.
29. Hasan SA, Jabeen S. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. Biotechnol Biotechnol Equip. 2015 Jan 2;29(1):45–53.
30. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H, et al. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi. BioMed Res Int. 2013;2013:Article number 371058.
31. Shukor MS, Shukor MY. Statistical diagnostic tests of the Luong model in fitting molybdenum reduction from the bacterium *Bacillus* sp. strain A.rzi. J Environ Microbiol Toxicol. 2014;2(2):53–7.