

Growth Inhibition Kinetics of Acetonitrile Biodegradation

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ABSTRACT

As the most pristine and one of the biggest continents in the southern hemisphere, Antarctica has over the decade accumulated hydrocarbon pollution mainly due to human activities related to logistics and transportation in this area. Acetonitrile spills caused by the sinking of the cargo ships call for research into acetonitrile-degrading microorganisms in the form of bioremediation in order to be ready for disasters in the future. The efficiency of a previously isolated acetonitrile-degrading sludge consortium as a bioremediation technique has been demonstrated. However, as the acetonitrile concentration rises, its growth was severely restrained. Acetonitrile's inhibitory effect on this consortium's development rate is modeled in this work using 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 acetonitrile was the Teissier-Edwards's model. The computed values for the Teissier constants like maximal reduction rate (μ_{max}), half saturation constant for maximal degradation (K_s) and half inhibition constant (K_i) were 0.934 1/H (95% confidence interval 0.301 to 1.567), 1.504 g/L (95% confidence interval 0.877 to 2.131), and 4.574 g/L (95% confidence interval 2.764 to 6.383), respectively. The parameters obtained from this study will be beneficial in acetonitrile biodegradation works.

INTRODUCTION

Organonitrile are a class priority pollutant that have a wide range of toxic effects on all levels of life, including humans. They are equally known to be extremely carcinogenic and mutagenic [1–3]. Acetonitrile, acrylonitrile, and benzonitrile are common examples of these compounds, which are widely used in laboratories and industries as solvents and extractants, or as an ingredient in pharmaceuticals, plastics, synthetic rubbers, drug intermediates (chiral synthons), herbicides and pesticides (e.g., dichlobenil, bromoxynil, ioxynil, butiril), and so on [4–6]. As a result, organonitrile compounds are frequently present in the effluents from these applications, which makes it difficult to degrade or detoxify these compounds in wastewater. Investigating technology and techniques that can efficiently treat these substances before they are safely released into the environment or combined with other wastewater for further

treatment is therefore of significant research and practical importance.

Ozone and photocatalytic oxidation are two examples of chemical treatments that can be used to remediate these pollutants, however due to their severe reaction conditions, production of secondary pollutants, and high operational costs, these approaches are frequently not the best option [7,8]. According to studies [9,10], the ecologically beneficial technology of bioremediation has the potential to remove these substances by converting them into harmless intermediates or, eventually, carbon dioxide and water. Many researchers have investigated the microbial degradation of acetonitrile and their derives chemicals using various isolates, such as *Nocardia rhodochrous* [11], *Pseudomonas putida* [3], *Rhodococcus* sp. N 774 [12], *Corynebacterium* sp. C5 [13], *Rhodococcus rhodochrous* PA-34 [14], *Pseudomonas marginalis* [15], *R. erythropolis* BL1 [16], *Rhodococcus erythropolis* A10 [17],

Cryptococcus sp. UFMG-Y28 [18], *Brevibacterium imperialis* CBS489-74 [19], *Candida guilliermondii* CCT 7202 [20], *Comamonas testosteroni* and *Acidovorax* sp. [21], *Paracoccus thiophilus* [22], *Kluyveromyces thermotolerans* MGBY 37 [23] and *Klebsiella oxytoca* [5]. Despite the fact that isolated microorganisms may have a promising potential to degrade the toxic organonitrile compounds, it may possibly be more practical to use a mixed culture (a consortium) for the remediation or degradation of these toxic chemicals.

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 growth-linked substrate utilization rate has been extensively described using the Monod equation [24,25]. The original Monod model, however, was inapplicable when a substrate, like acetonitrile, showed strong inhibition toward its own biodegradation. Instead, a derivative of this model with additional constants that offered substrate corrections have been developed. For this work, a number of microbial growth and biodegradation kinetic models are available. Numerous literatures generalize the use of the Haldane model to model substrate inhibition to growth or degradation rate. This is despite the fact that several other models have been shown to be more accurate for a single substrate-inhibiting compound, such as phenol. Aside from the commonly reported Haldane model [26], numerous other models such as Edward [27] and Luong [28,29] have been found to be optimal. As a result, in some cases using comprehensive models instead of the Haldane might be appropriate. The exclusive use of the Haldane model should not be applied indiscriminately without properly fitting these other models to the available growth or degradation rate data and appropriate statistical evaluation.

The growth kinetics of an acetonitrile-degrading consortium was not previously determined using various inhibitory growth kinetic models. This research is being done to assess how well these models can be used to predict the effect of acetonitrile on this consortium's growth rate.

MATERIALS AND METHODS

Source of data

The software Webplotdigitizer 2.5 [40], which digitizes figures and has been widely used and praised for its dependability [41–44] was used to analyze previously published data [45].

Fitting of the data

Nonlinear regression with a Marquardt algorithm that minimizes sums of squares of residuals was used to fit the nonlinear equations to the growth data using CurveExpert Professional software (Version 1.6). The goal of this search strategy is to reduce the sum of the squares of the variations between the predicted and measured values.

Statistical analysis

Using the same set of experimental data, models with different numbers of parameters were compared to one another to see if there was a significant difference in terms of fitness. Statistics functions such as adjusted coefficient of determination (R^2), Root-Mean-Square Error (RMSE), corrected AICc (Akaike Information Criterion), bias factor and accuracy factor (BF, AF), were used. **Table 1** shows the various growth inhibition kinetics models that are available.

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

Author	Degradation Rate	Author
Monod	$\frac{\mu_{max}S}{S + K_s}$	[30]
Haldane	$\frac{\mu_{max}S}{S + K_s + \left(\frac{S^2}{K_i}\right)}$	[31]
Teissier	$\mu_{max} \left(1 - \exp\left(-\frac{S}{K_i}\right) - \exp\left(\frac{S}{K_s}\right) \right)$	[32]
Aiba	$\mu_{max} \frac{S}{K_s + S} \exp\left(-\frac{S}{K_i}\right)$	[33]
Yano and Koga	$\frac{\mu_{max}S}{S + K_s + \left(\frac{S^2}{K_i}\right) \left(1 + \frac{S}{K}\right)}$	[34]
Han and Levenspiel	$\mu_{max} \left(1 - \left(\frac{S}{S_m}\right)^n \left(\frac{S}{S + K_s \left(1 - \left(\frac{S}{S_m}\right)^m\right)} \right) \right)$	[35]
Luong	$\mu_{max} \frac{S}{S + K_s} \left(1 - \left(\frac{S}{S_m}\right)^n \right)$	[36]
Moser	$\frac{\mu_{max}S^n}{K_s + S^n}$	[37]
Webb	$\frac{\mu_{max}S \left(1 + \frac{S}{K}\right)}{S + K_s + \frac{S^2}{K_i}}$	[38]
Hinshelwood	$\mu_{max} \frac{S}{K_s + S} (1 - K_p P)$	[39]

Note:
 μ_{max} maximal specific growth rate
 K_s half saturation constant
 K_i inhibition constant
 S_m maximal concentration of substrate tolerated
 K_p product inhibition constant
 m, n, K curve parameters
 S substrate concentration
 P product concentration

Equation 1 was used to determine the RMSE, which is a penalty for having too many parameters. Here, n represents the number of experimental data, p represents the number of parameters generated by the model, and the experimental data and values projected by the model are, respectively, O_{bi} and P_{di} [46].

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (P_{di} - O_{bi})^2}{n-p}} \quad (\text{Eqn. 1})$$

In linear regression, the best fitting model was determined by R^2 or coefficient of determination. However, in nonlinear regression, the R^2 does not give a comparative analysis where the number of parameters between models is different. To overcome this, adjusted R^2 was used to calculate the quality of the nonlinear models. In the adjusted R^2 formula, S_y^2 is the total variance of the y-variable and RMS is Residual Mean Square.

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

$$Adjusted (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \quad (\text{Eqn. 3})$$

The Akaike Information Criterion (AIC) can be used to compare the relative quality of various statistical models for a

given set of experimental data. Instead, for data sets with many parameters or few values, the corrected AIC, AICc, should be used [47]. The following Eqn. 4 was used to compute the AICc.

$$AICc = 2p + n \ln \left(\frac{RSS}{n} \right) + 2(p + 1) + \frac{2(p+1)(p+2)}{n-p-2} \quad (\text{Eqn. 4})$$

AICc provides details on the differences in parameter counts and model fitting between two models. The best match between the models would be shown by the AICc value that is the least [47]. Another statistical approach based on information theory, in addition to AICc, is the Bayesian Information Criterion (BIC) (Eqn. 5). More so than AIC, this error function penalizes the quantity of parameters [48].

$$BIC = n \cdot \ln \frac{RSS}{n} + k \cdot \ln(n) \quad (\text{Eqn. 5})$$

A further error function method based on the information theory is the Hannan–Quinn information criterion (HQC) (Eqn. 6). The HQC is strongly consistent unlike AIC due to the $\ln \ln n$ term in the equation [49]

$$HQC = n \times \ln \frac{RSS}{n} + 2 \times k \times \ln(\ln n) \quad (\text{Eqn. 6})$$

The models' goodness-of-fit was evaluated using BF and AF. To achieve a perfect match between the predicted and observed values in biodegradation, the Bias Factor should be set to 1. A Bias Factor (Eqn. 7) value greater than one indicates a fail-safe model, while a Bias Factor less than one indicates a fail-negative model. The value of Accuracy of 1 indicates a less precise prediction (Eqn. 8).

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

$$\text{Accuracy factor} = 10 \left(\sum_{i=1}^n \log \frac{|(Pd_i/Ob_i)|}{n} \right) \quad (\text{Eqn. 8})$$

RESULTS AND DISCUSSION

In this work, ten distinct growth models (Figs. 1 to 6) were utilized to fit the experimental data of [45]. The Monod model's flaw is that it ignores the unique regulatory complexity, variable response to environmental circumstances, and ability of bacteria to produce a variety of products and by-products in their natural metabolism. The Teissier model, which had the greatest adjusted R² values, the lowest values for RMSE and AICc, the lowest F-test values, and the Bias Factor and Accuracy Factor values that were closest to unity (1.0), was the most accurate and statistically significant of the kinetic models used (Table 2). Neither did the Aiba, Han-Levenspiel, Moser, nor Monod models converge.

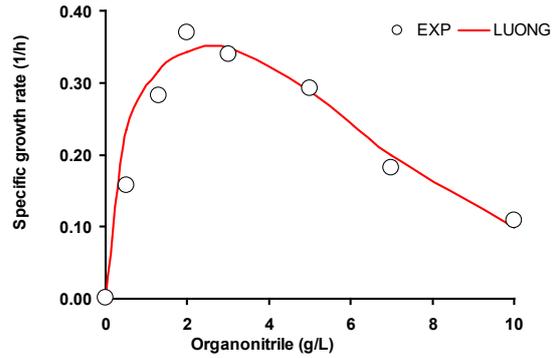


Fig. 1. Substrate inhibition kinetic on acetonitrile as modelled using the Luong model.

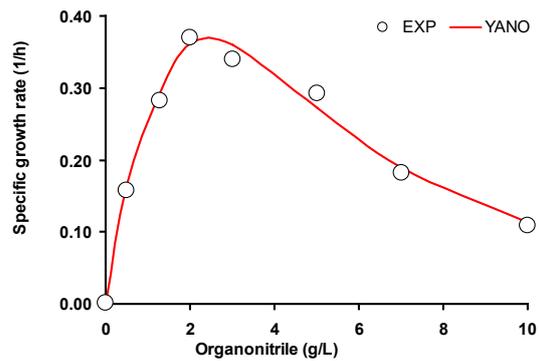


Fig. 2. Substrate inhibition kinetic on acetonitrile as modelled using the Yano model.

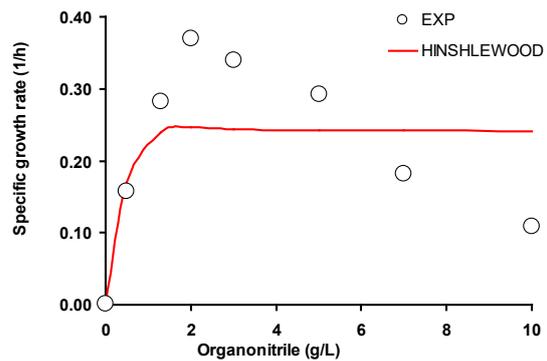


Fig. 3. Substrate inhibition kinetic on acetonitrile as modelled using the Hinshelwood model.

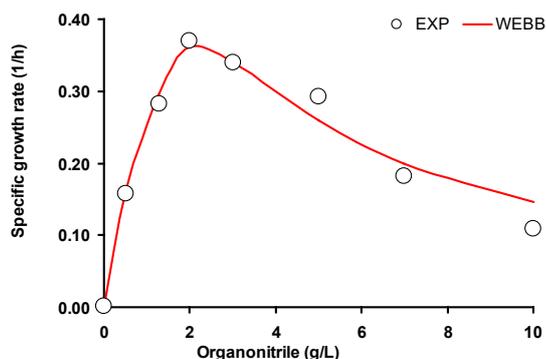


Fig. 4. Substrate inhibition kinetic on acetonitrile as modelled using the Webb model.

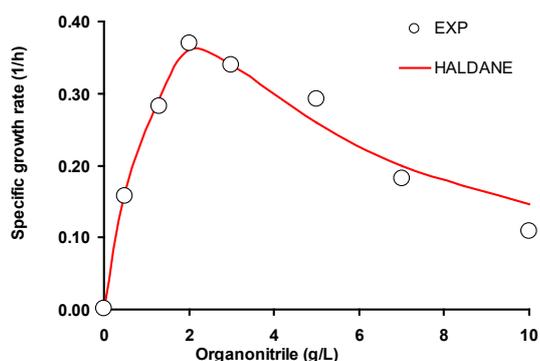


Fig. 5. Substrate inhibition kinetic on acetonitrile as modelled using the Haldane model.

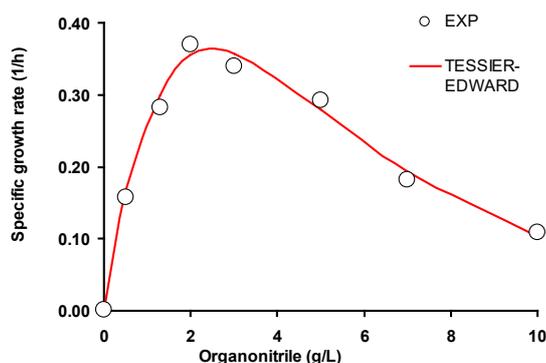


Fig. 6. Substrate inhibition kinetic on acetonitrile as modelled using the Teissier-Edward model.

Table 2. Statistical analysis of the various fitting models.

Model	p	RMSE	adR ²	AICc	BIC	HQC	BF	AF
Luong	4	0.05	0.82	-6.91	-46.59	-49.05	1.06	1.11
Yano	4	0.02	0.98	-23.26	-62.95	-65.41	1.01	1.04
Teissier-Edward	3	0.02	0.98	-43.21	-64.31	-66.16	1.01	1.05
Aiba	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Haldane	3	0.02	0.95	-35.75	-56.85	-58.69	1.04	1.08
Monod	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Han and Levenspiel	5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Moser	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hinshlewood	4	0.11	n.a.	7.27	-32.41	-34.87	1.01	1.32
Webb	4	0.03	n.a.	-16.20	-55.88	-58.34	1.04	1.08

Note:
 p no of paramaters
 RMSE Root Mean Square Error
 Ra² Adjusted Coefficient of determination
 BF Bias factor
 AF Accuracy factor
 n.a. not available (model not converged)

The computed values for the Teissier constants like maximal reduction rate (μ_{max}), half saturation constant for maximal degradation (K_s) and half inhibition constant (K_i) were 0.934 1/h (95% confidence interval 0.301 to 1.567), 1.504 g/L (95% confidence interval 0.877 to 2.131), and 4.574 g/L (95% confidence interval 2.764 to 6.383), respectively. However, the genuine μ_{max} should occur where the gradient for the slope is zero, and in this case, the μ_{max} value produced by curve fitting interpolation was around 0.363 1/h at 2.49 g/L acetonitrile. The Teissier equation using the values obtained from fitting is as follows;

$$\mu = 0.363 \left(1 - \exp\left(-\frac{S}{4.574}\right) - \exp\left(\frac{S}{1.504}\right) \right)$$

Due to the shortcomings of earlier models like Haldane, Andrews and Noack, Web, and Yano, which were unable to explain some rare scenarios when growth rate turned zero at very high substrate concentration, models like Luong, Teissier, and Hans-Levenspiel were developed [50]. In some circumstances, at high substrate concentrations, the substrate itself, as a result of its repressive and toxic properties, inhibits microbial growth rate. To date, the majority of the Luong model reported for microbial degradation of xenobiotics has focused on phenol-degrading bacteria [28,51,52] and molybdenum-reducing consortium [53,54]. Perhaps, this the first work to model the degradation of acetonitrile using bacterial consortium.

CONCLUSION

A complete cessation of maximum growth to the growth rate of the Antarctic Sludge Consortium was observed in this study, and the use of various kinetics models in coupled with a thorough statistical treatment of the model suggests that the Luong model was superior to the widely used Haldane model in fitting the growth rate at various acetonitrile concentrations. The maximum substrate concentration at which growth rate completely stops can be predicted using the Luong model.

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