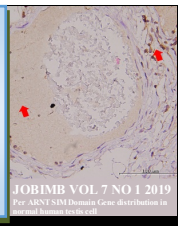
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Kinetics of Cyanide Degradation by *Azotobacter vinelandii*

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HISTORY

Received: 16th March 2019
Received in revised form: 17th of April 2019
Accepted: 10th of May 2019

KEYWORDS

cyanide
degradation
Azotobacter vinelandii
Modelling
Aiba

ABSTRACT

Cyanide is extremely toxic to living organisms. It is utilized in industries for gold and silver extraction, pharmaceuticals, plastic processing, electroplating, and agricultural chemistry. Cyanide can persevere for a long time in the soil and its bio-degradation is the best economical practice. A formerly isolated cyanide-degrading bacterium *Azotobacter vinelandii* exhibited substrate inhibition to degradation rate. Significant degradation inhibition constants were achieved reliably by means of non-linear regression modeling of the degradation rate profile utilizing models for substrate inhibition like Haldane, Teissier-Edward, Monod, Yano and Koga, Luong, Edward (Webb) Luong and Aiba models. Aiba model was selected as the top model established on statistical assessments like root mean square error, adjusted coefficient of determination, bias factor and accuracy factor. The calculated values for the Aiba constants q_{max} (the maximum specific substrate degradation rate (h^{-1}), K_s (concentration of substrate at the half maximal degradation rate (mM) and K_i (inhibition constant (mM)) were 0.060 (95% CI, 0.024 to 0.096), 0.302 (95% CI, 0.381 to 0.222) and 0.953 (95% CI, 0.568 to 1.338), respectively. The novel constants gotten from the modeling application can be valuable for advanced secondary modeling implicating the influence of media conditions and some other dynamics on cyanide biodegradation by this bacterium.

INTRODUCTION

Cyanide is a damaging, lethal and noxious waste that portends danger to the environment and animal well-being. It is extensively used in various industries such as mining, electroplating, photoprocessing, gas production, fertilizer production, pharmaceuticals and plating [1]. The presence of cyanides in sewages poses a serious danger and thus, must be eliminated from all sewages before to release to the environment [2]. The existence of cyanides in water bodies causes extensive death of aquatic animals and upsets microorganisms' in water. Moreover, it is lethal and harmful to humans as it causes neurological effects such as quick breathing, tremors, loss of weight, injury in the nerves and many other diseases. Cyanide contact with the skin may result to irritation and sores [3]. Various methods of treating sewages containing cyanides are used which includes chemical oxidation, physical and biological processes [4]. These chemical approaches are sometimes not economically/environmentally friendly as a result of the use of hazardous chemical agents unlike bioremediation. Besides, they create toxic residues and cannot totally eliminate all complexes of cyanide [1,7]. Biological methods are reported to be most efficient method of cyanide removal. For instance, Simsek [1] carried out column and batch research with a Purolite resin so as to get rid of cyanide ions from aqueous solutions. Furthermore, Hijosa-Valsero [8] examine plasma discharge technology for removal of cyanide from water having a concentration of 1 ppm. Akcil in 2003 examined the biodegradation of cyanide by a certain bacteria [9].

These microorganisms could proficiently convert cyanide into compounds that are less toxic, such as ammonia and carbonate [9]. Resting cells of *Serratia marcescens* was reported to biodegrade cyanide using shake flask as bioreactor [10,11].

Moreover, several bioremoval techniques for cyanide were presented in a cyanide biological review [12]. Incidentally, for purification of water to reach qualified requirements, nanotechnology have been developed as a new tool [13,14]. Consequently, it is imperative to observe the proliferation of microbes, their ability to various levels of cyanide as a substrate and the part they take in the overall efficacy of a biodegrading process. This requires some evaluation through kinetic studies [15]. Various kinds of kinetic models were explained in literature hence, a brief account of some of these models was used in this study as illustrated below.

The Haldane model was first introduced in 1930 and is the leading and well recognized model for Substrate Inhibition Kinetics owing to its influence which makes it to be conventionally used by a lot of scientists [16–18]. It utilises the equation to clarify enzyme inhibition via the creation of an inactive enzyme complex with twofold substrate molecules. The Haldane equation was established by a number of researchers to provide an appropriate fit of specific growth rate (μ) or specific degradation rate (q) plot against Half Saturation Coefficient (S) for growth at high levels of substrates as shown in the equation. As substrate inhibition constant (K_i) nears infinity the equation nears the familiar quadrilateral hyperbola for the Monod connexion [19]. Where K_i is the substrate inhibition constant.

This kinetic model was designed by Tessier in 1942 because of the theory of diffusion-controlled substrate as derived in the equation. It is an exponential kinetic model. In several biotechnological processes, higher substrate concentrations or its products frequently results in inhibitory effects, which several times results to poor substrate utilisation. Additionally it reduces the yield of the product and fermentation rates, affecting metabolic reactions of microorganisms if administered at higher level [15,20].

Monod's model correlates the growth rate of the bacteria to substrate concentration of a specific growth controlling substrate as revealed in the equation [21]. This is about the most broadly used model for modelling growth kinetics. Though, it has some imperfections in some cases where unusual *Km* values could be gotten due to multi-S-constraint endogenous metabolism, high cell concentration, internal transport and ionic strength limitations non-stationery processing, product inhibition and biosorption [22].

The Yano and Koga model is offered by Yano and Koga and depends on the theoretical experiment on the dynamic performance of solitary flask continuous fermentation system dependent on the growth inhibition at higher concentration to rate limiting substrate as depicted in the equation [23]. For example; acetic acid fermentation from ethanol and gluconic acid fermentation from glucose etc [18].

The Edward (Webb) kinetic model was proposed as a modified form of Haldane Model of 1930. The model was found to be less effective compared to the latter [18]. Luong offered the utilisation of substrate inhibition to the microbial growth, relating butanol inhibition on the proliferation of yeast [24]. The model is very important in substrate inhibition kinetics. Nonetheless, the model is the extensive Monod's Model that deduces substrate stimulation at lower and higher concentrations. This model can predict the maximum substrate concentration value (S_m), if the exceeded growth is completely inhibited [19]. The kinetic model was offered by Aiba in order to suggest a model for Microbial growth [25]. It illustrates an exponential model that compares growth inhibition values with substrate degradation. The equations for all the models are shown in **Table 1**.

Conversely, kinetics modelling illustrating substrate inhibition to substrate degradation rate has reported in literatures [26–28]. Kinetics modelling for Biodegradation of Ferrous II cyanide complex ions by *Pseudomonas fluorescens* in a packed bed column reactor was reported [29]. Biodegradation kinetics of cyanide by *Serratia marcescens* strain AQ07 was reported [30]. Furthermore, biodegradation kinetics of cyanidase by the use of enzyme immobilised procedures in the form of powder that is fitted to a batch reactor and kinetically modelled by the use of simple Michaelis–Menten equation was also reported [31]. These are the major available literatures that reported growth kinetics inhibition of bacteria using cyanide as a substrate. Hence, the objective of this report is to assess the kinetic model that better fits the bioremoval capacity of *Azotobacter vinelandii* with the aid of upgrading the process.

Table 1. A number of mathematical models established for degradation kinetics encompassing inhibition of substrate.

Mathematical Models	Rate of Degradation	Author
Haldane	$q_{\max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[32]
Teissier	$q_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[33]
Monod	$q_{\max}\frac{S}{K_s+S}$	[21]
Yano and Koga	$\frac{q_{\max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[23]
Edward (Webb)	$q_{\max}\frac{S\left(1+\frac{S}{K}\right)}{S+K_s+\left(\frac{S^2}{Ki}\right)}$	[33]
Luong	$q_{\max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[19]
Aiba	$q_{\max}\frac{S}{K_s+S}\exp\left(-K_pP\right)$	[34]

Note:

q_{max}	maximal rate of degradation (h^{-1})
K_s	half saturation constant for maximal reduction (ppm)
S_m	maximal substrate concentration tolerated and (ppm)
m, n, K	curve parameters
S	concentration of substrate (ppm)

MATERIALS AND METHODS

Acquirement and Fitting of Data

The data tabulated in Table 1 [34] were used in this work. The primary and secondary (inhibition kinetics) degradation were built-in utilizing a nonlinear regression that operate a Marquardt algorithm Curve Expert Professional software (Version 1.6), that reduces the sums of square of the variances among results of the projected and measured data. The specific rate of degradation (**Eqn. 1**) was modelled in accordance with the no lag phase Huang model (**Fig. 1**) [35,36].

$$y = A + y_{max} - \ln\left(e^{A} + \left(e^{Y_{max}} - e^{A}\right)e^{-\mu_m B(x)}\right)$$

and

$$B(x) = x + \frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}} \qquad \textbf{(Eqn. 1)}$$

Statistical analysis

Different statistical procedures like the adjusted coefficient of determination (R^2), root-mean-square error (RMSE), accuracy and bias factors were employed [37]. The RMSE was calculated in line with Eqn. 2, where Ob_i are the experimental data, p is the number of factors of the evaluated model, Pd_i are the predicted values by the model and n is the number of experimental data.

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

Evaluations of the adjusted R^2 value are conducted in line with equations **Eqns. 3 and 4** respectively where RMS is Residual Mean Square and s_y^2 is the absolute variance of the y-variable.

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

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

Goodness-of-fit tests for the models; Bias and Accuracy Factors (BF) and (AF) was presented by Ross [38] as illustrated in **Eqns. 5 and 6** respectively'

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

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

RESULTS AND DISCUSSION

The statistical study in **Table 2** illustrates that the best model was Aiba. The bulk of the statistical evaluation like the lowest figures for RMSE, the maximum adjusted R^2 values and with Bias and Accuracy Factors closest to unity (1.0) showed that it is the best model (**Figs. 2 to 7**).

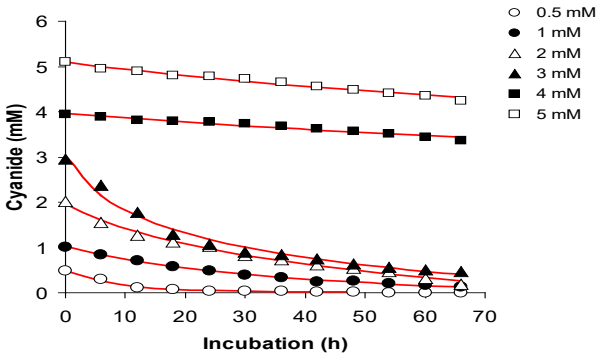


Fig. 1. Cyanide degradation by *A. vinelandii* as modelled according to the no lag phase Huang model (solid lines).

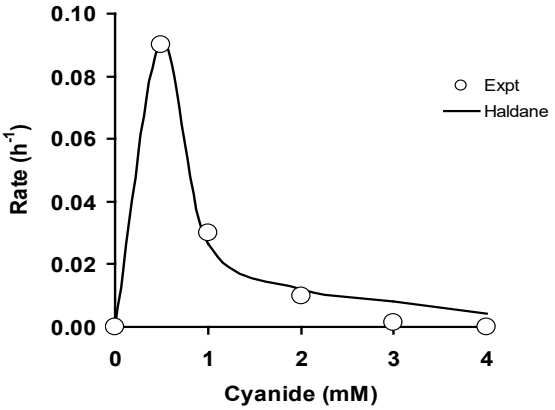


Fig. 2. The effect of cyanide concentration on the specific rate of degradation by *A. vinelandii* as modelled according to the Haldane model.

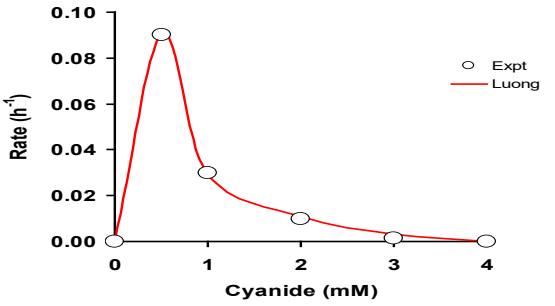


Fig. 3. Effect of cyanide concentration on the specific rate of degradation by *A. vinelandii* as modelled utilizing Luong model.

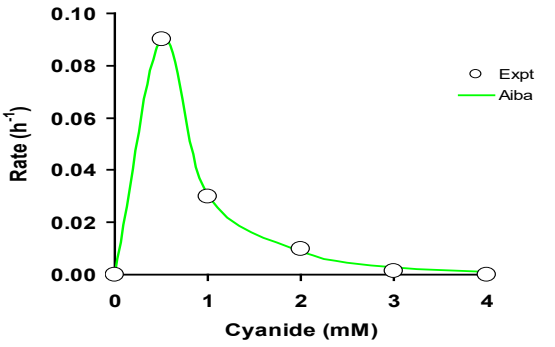


Fig. 4. The effect of cyanide concentration on the specific rate of degradation by *A. vinelandii* as modelled according to the Aiba model.

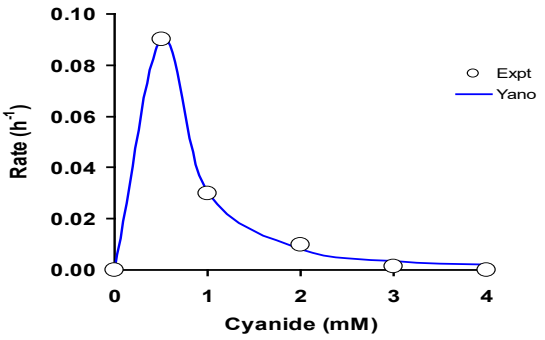


Fig. 5. The influence of cyanide concentration on the specific rate of degradation by *A. vinelandii* as modelled utilizing Yano and Koga model.

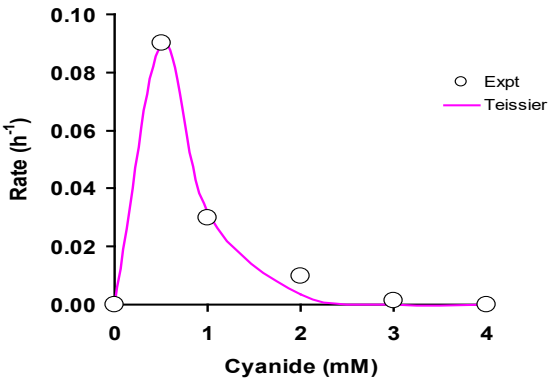


Fig. 6. The effect of cyanide concentration on the specific degradation rate of *A. vinelandii* as modelled according to the Teissier model.

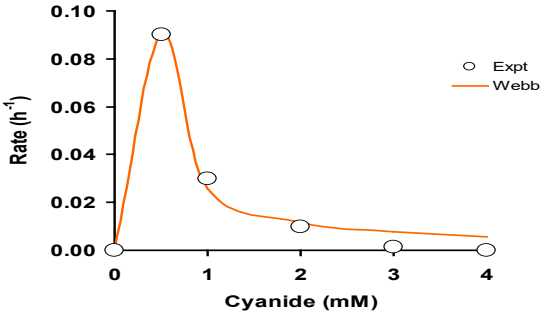


Fig. 7. The influence of cyanide concentration on the specific rate of degradation by *A. vinelandii* as modelled in line with Edward (Webb) model.

Table 2. Statistical Evaluation of the Degradation Kinetic Models.

Model	p	RMSE	adR^2	AICc	BF	AF
Luong	4	0.002	0.997	-18.93	1.12	1.13
Yano	4	0.002	0.994	-14.72	1.10	1.17
Tessier-	3	0.004	0.979	-46.87	0.55	1.84
Aiba	3	0.001	0.999	-65.79	1.07	1.12
Haldane	3	0.005	0.968	-44.77	1.26	1.31
Webb	4	0.006	0.952	-0.73	0.00	0.00

Note:
SSE Sums of Squared Errors
RMSE Root Mean Squared Error
R^2 Coefficient of Determination
adR^2 Adjusted Coefficient of Determination
BF Bias Factor
AF Accuracy Factor

The calculated values for the Aiba constants q_{max} (the maximum specific substrate degradation rate (h^{-1}), K_s (concentration of substrate at the half maximal degradation rate (mM) and K_i (inhibition constant (mM)) were 0.060 (95% CI, 0.024 to 0.096), 0.302 (95% CI, 0.381 to 0.222) and 0.953 (95% CI, 0.568 to 1.338), respectively. In a previous study, the Luong model was found to be the best model in fitting the effect of cyanide to the cyanide-degradation rate of *Serratia marcescens* strain AQ07 [30].

A lot of xenobiotics biodegradation works utilise substrates that hinders microbial proliferation or biodegradation of substrate because of the noxiousness of the substrates. These includes halogenated and aromatic hydrocarbons and other elemental biotransformation procedures that comprise metals like chromium, mercury and molybdenum [39–41]. As a result of this occurrence the Monod model fails to explain the degradation, growth profile. Other models like Wayman and Tseng [42], Haldane, Han-Levenspiel, Luong, Andrews &Noack, and Webb can be utilised [43]. Additional inhibitory kinetics model was suggested by Aiba et al. [34] which involves product inhibition kinetics. The model was improved afterwards to take up a one-way mechanism for both the product and substrate inhibition rate by Edwards [33]. This improved version (Aiba–Edwards model) was described as the best model suitable for degradation of phenol by *Ralstonia eutropha* [44] and the inhibitory result of sodium, chloride (NaCl) on the halo tolerant *Kocuria rosea* [45]. The modified Aiba-Edwards model was not able to perfect substrate inhibition degradation kinetics for a lot of xenobiotics but its utilisation in relating the effect of cyanide concentration to degradation rate in this study is novel.

CONCLUSION

The cyanide biodegradation displayed standard substrate inhibition to the rate of degradation. This biodegradation kinetics can be modelled utilising different models in various literatures. Considering the various models utilised to explain effect of substrate to the degradation rate, the modified Aiba-Edwards model was excellent based on statistical computations. It is the first time the model was exploited to perfect the influence of substrate on cyanide biodegradation rate.

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