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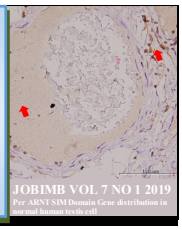

Kinetics of Caffeine Inhibition to the Growth of *Caulobacter crescentus* on Caffeine

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HISTORY

Received: 28th March 2019
Received in revised form: 26th of April 2019
Accepted: 20th of July 2019

KEYWORDS

caffeine
Caulobacter crescentus
mathematical modelling
maximum specific growth rate
Haldane

ABSTRACT

Caffeine is a purine alkaloid naturally found in many species of plant and can be degraded by bacteria. Prolong caffeine consumption is well-known to have serious adverse effects. A caffeine-degrading bacterium had been shown to be inhibited by high concentration of caffeine. To study this phenomenon in greater detail, the effect of caffeine on the maximum specific growth rate on caffeine is studied using various inhibition kinetic models such as Luong, Haldane, Aiba, Yano, Teissier, Webb and Monod. The Haldane model was the best model in modelling the effect of caffeine concentration to *Caulobacter crescentus* maximum specific growth rate based on statistical tests such as corrected AICCc (akaike information criterion), bias factor (BF), adjusted coefficient of determination (R^2) and root-mean-square error (RMSE). Parameters obtained from the fitting exercise were maximum specific growth rate (q_{max}), K_s (concentration of substrate at the half maximal specific growth rate (mg/L) and K_i (inhibition constant) mg/L with their values at 0.7620 (95%, C.I. or confidence interval from 0.603 to 0.920), 0.1050 mM (95% C.I. from 0.001 to 0.211) and 4.614 mM (95% C.I. from 2.154 to 7.073), respectively. These biologically meaningful coefficients will be useful in predicting growth conditions under batch studies and also probably under actual bioremediation strategy where the concentration of caffeine in the treatment may need be diluted to nontoxic concentration prior to remediation.

INTRODUCTION

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) is an alkaloid whose basic structure is purine and found commonly in many plant species. Amongst them, coffee, guarana, yerba mate, colanut, chocolates, tea and cocoa beans are the most common known [1,2]. As such, it is a human major dietary ingredient found in most non-alcoholic beverages, food products and also in pharmaceuticals due to stimulatory and muscle relaxant properties [3,4]. Continuing caffeine intake can lead to osteoporosis, cardiac arrhythmias, adrenal stimulation, inhibition of DNA repair mechanism, fatigue, headache, complications in pregnant women aging as it upsurges the risk of abortion and affects fetal growth therefore causing malfunction in the fetus [5–8]. Apart from the adverse effect, degradation caffeine is paramount from the environment point of view. Caffeine effluents and by-products generated from tea and coffee industries comprise major part of the agro-industrial wastes in coffee producing countries [9]. Although these wastes are rich in carbohydrates and proteins, but they cannot be used in animal feds because of the presence of toxic caffeine, other anti-nutritional factors such as polyphenols, tannins and other detrimental substances [10,11]. Furthermore, turning these wastes to neighbouring water bodies would affect the aquatic animals living [12,13]. Caffeine present in soil affects soil fertility as it impedes seedlings growth and seed germination [14]. Thus, from both environmental and medical view, caffeine degradation is a major issue in coffee processing industries.

Caffeine degradation using conventional techniques (supercritical fluid water and solvent extraction methods) are non-specific, expensive and toxic to caffeine. More so, it involves the use of toxic substances that are toxic environment. Enzymatic and microbial techniques of caffeine degradation are recommended in order to overcome the conventional method [15]. Recently, a better alternative to this problem is the use of microbial techniques in caffeine degradation as it is easier, cheaper, economic, reduce time management, eco-friendly, specific and disease free [16–18]. Microbial isolates capable of caffeine degradation has been well deliberated in *Trichosporon*

asahii, Leifsonia, Klebsiella, Serratia, Pseudomonas, Caulobacter crescentus inter alia. Unlike in higher organisms, bacteria can utilise caffeine as a sole source of nitrogen and carbon for their growth [19,20]. Caffeine is known to inhibit bacterial growth even in caffeine-degrading microorganisms. In the degradation of caffeine by the bacterium *Leifsonia* sp. strain SIU, inhibition kinetic analysis shows that the specific growth rate was severely inhibited at high caffeine concentration culminating in the Luong model being the best model to fit the inhibition curve [4].

This work is aimed at studying the bacterial growth kinetics of caffeine reduction by the novel caffeine-degrading bacterium *Caulobacter crescentus*, which was isolated using six different kinetic models [20]. The best model to represent the bacterial growth kinetic was assessed through the verification of the fitting models using different statistical approaches, such as the corrected AICc (Akaike Information Criterion), F test, accuracy factor (AF), bias factor (BF), adjusted coefficient of determination (R^2) and the Root-Mean-Square Error (RMSE).

MATERIALS AND METHODS

Data

Data previously sourced from Fig 1. [20] was modelled using primary models to find the best models and Huang was discovered to be the best model [21].

Fitting of the data

Fitting of the bacterial growth curve using various growth kinetic models (**Table 1**) was carried out using the CurveExpert Professional software (Version 1.6) by nonlinear regression utilizing the Marquardt algorithm. q_{max} of estimation was carried out by the steepest ascent rifle of the curve while the crossing of this line with the x-axis is an estimation of λ.

Statistical analysis

Statistical significant difference between the models was calculated through various methods including the adjusted coefficient of determination (R^2), accuracy factor (AF), bias factor (BF), Root-Mean-Square Error (RMSE) and corrected AICc (Akaike Information Criterion) as before [29]. The adjusted coefficient of determination (R^2) was calculated according to Eqn. 1

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

The root-mean-square error (RMSE) was calculated according to Eqn. 2, where *p* is the number of parameters of the assessed model, *Pdi* are the values predicted by the model, *Obi* are the experimental data and *n* is the number of the experimental data.

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

The Alaike information criterion (AIC) with correction was used following (AICc). The AICc was calculated for each set of data and for each model according to Eqn. 3, where RSS is the residual sum-of-squares, n is the number of data points and p is the number of parameters of the model.

$$AICc = 2p + nln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \qquad \textbf{(Eqn. 3)}$$

Ross and McMeekin, [30] has introduced other tests for the goodness-of-fit of the models; Bias Factor (BF) and Accuracy Factor (AF). The equations are as follows:

$$\text{Accuracy Factor} = 10^{\left\{\sum_{i=1}^{n} log^{\frac{|(Pdi/Obi)|}{n}}\right\}} \qquad \textbf{(Eqn. 4)}$$

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

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

Author	Degradation Rate	Author
Monod	$q_{max}\frac{S}{K_s+S}$	[22]
Haldane	$q_{max}\frac{S}{S+K_s+\frac{S^2}{K_i}}$	[23]
Teissier	$q_{max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right)$	[24]
Aiba-Edward	$q_{max}\frac{S}{K_s+S}\exp\left(\frac{-S}{K_i}\right)$	[25]
Yano and Koga	$\frac{q_{max}S}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[26]
Edward (Webb)	$q_{max}\frac{S\left(1+\frac{S}{K}\right)}{S+K_s+\left(\frac{S^2}{Ki}\right)}$	[27]
Luong	$q_{max}\frac{S}{S+K_s}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[28]

Note:

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

RESULTS AND DSICUSSION

The fitting to the curve representing the effect of caffeine on the specific growth rate on caffeine shows visually acceptable fitting except for the Monod model (**Figs. 1** to **7**). The best performance was found to be Haldane model with the lowest value for RMSE, AICc and the highest value for adjusted R2. The AF and BF values were also excellent for the model with their values were the closest to 1.0. The poorest performance was Monod (**Table 2**).

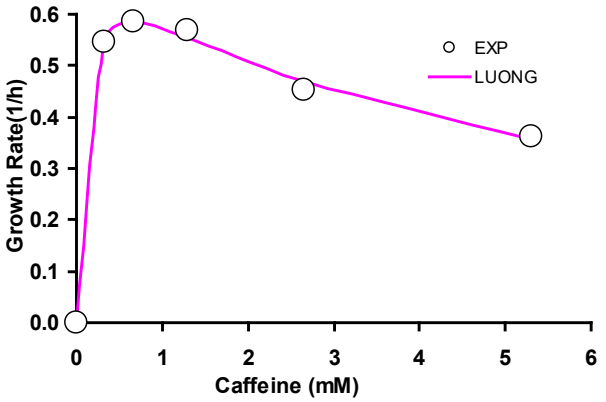

Fig. 1. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Luong model.

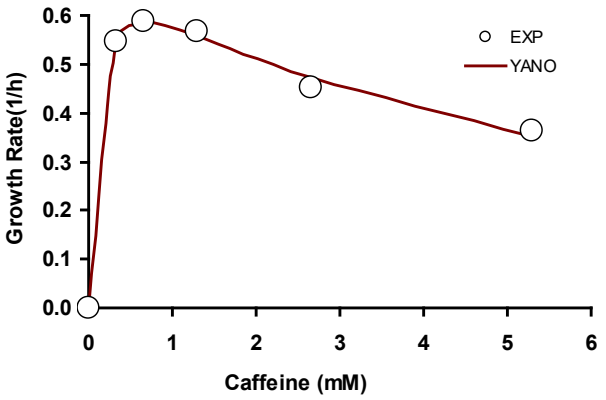

Fig. 2. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Yano model.

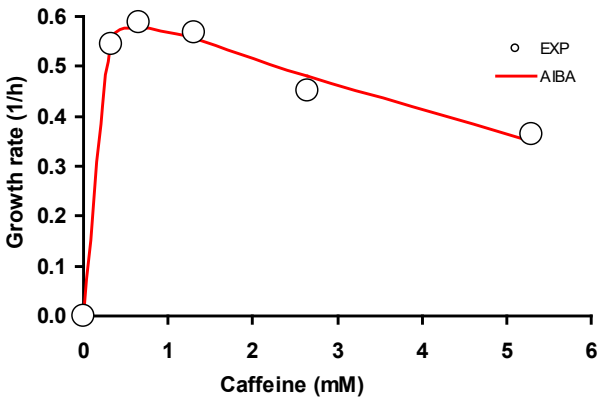

Fig. 3. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Aiba model.

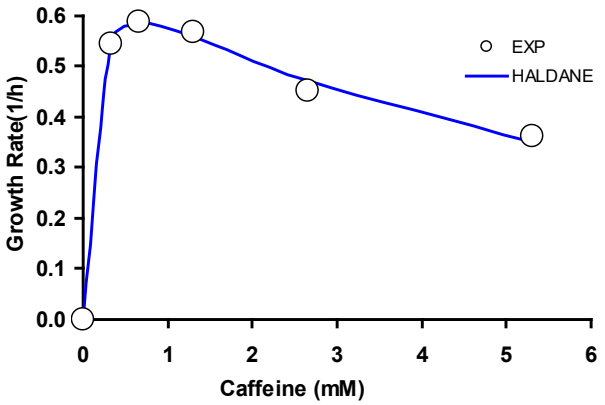

Fig. 4. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Haldane model.

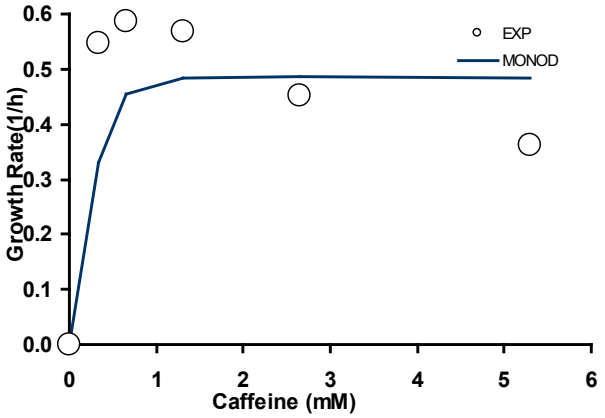

Fig. 5. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Monod model.

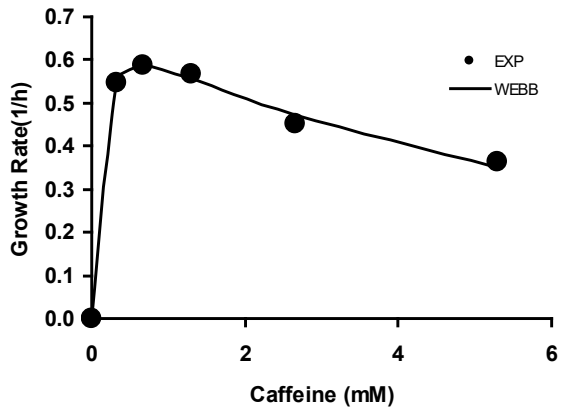

Fig. 6. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Webb model.

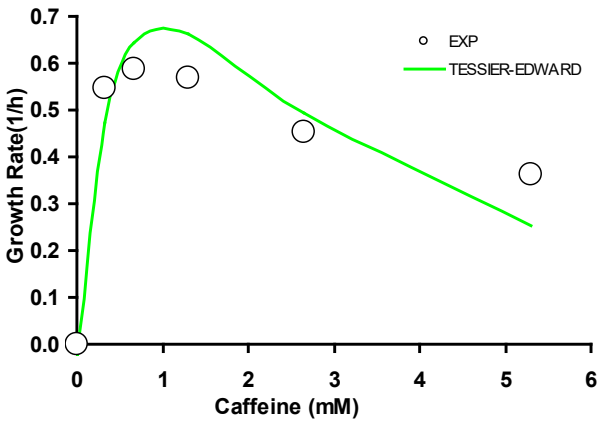

Fig. 7. The effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus* as modelled according to the Teissier model.

Table 2. Error function analysis on the models utilized to fit the curves of the effect of caffeine concentration on the specific growth rate of *Caulobacter crescentus*.

Model	p	RMSE	$AdjR^2$	AICc	BF	AF
Luong	4	0.014	0.993	11.84	1.000	1.013
Yano	4	0.014	0.992	12.73	0.999	1.014
Tessier-Edward	3	0.091	0.815	-3.54	0.975	1.127
Aiba	3	0.018	0.989	-26.02	0.999	1.022
Haldane	3	0.012	0.995	-31.27	0.999	1.014
Monod	2	0.070	0.834	-21.61	1.009	1.097
Webb	4	0.014	0.992	12.74	0.999	1.014

Note:

p	No of parameter
RMSE	Root Mean Squared Error
R^2	Coefficient of Determination
$AdjR^2$	Adjusted Coefficient of Determination
BF	Bias Factor
AF	Accuracy Factor

Most of the models showed good fitting as observed by eye with the exception of Monod model as shown from the Figs. Parameters obtained from the fitting exercise were maximum specific growth rate (q_{max}), K_s (concentration of substrate at the half maximal specific growth rate (mg/L) and K_i (inhibition constant) mg/L, with their values at 0.7620 (95%, C.I. or confidence interval from 0.603 to 0.920), 0.1050 mM (95% C.I., from 0.001 to 0.211) and 4.614 mM (95% C.I. from 2.154 to 7.073), respectively.

The modelling result shows that caffeine is toxic to bacterial growth resulting in a decrease in the maximum specific growth attained as the concentration of caffeine was increased. This finding is in compliance with the result obtained by Ahmad et al. [31] who reported Haldane as the best model for diesel degradation kinetics by *Burkholderia* sp. strain DRY27 and contrary with the results obtained by Gokulakrishnan and Gummadi [32] and Ibrahim et al. [4] who reported Luong as the best model for caffeine growth kinetics by *Pseudomonas* sp. GSC 1182 and *Leifsonia* sp. strain SIU respectively.

Due to the toxic effect of substrates, most studies on biodegradation of pollutants use substrate that impedes microbial growth and degradation. These substrates include halogenated and aromatic hydrocarbons and even elemental biotransformation processes that include metals such as molybdenum, copper, lead, mercury, chromium among others [33–35]. Underneath all these condition Monod model failed to depict the growth degradation profiles. Other models like Webb, Han-Levenspiel, Luong, Haldane and Andrews and Noack can also be used [36].

CONCLUSION

In conclusion, the Haldane model was the best model in modelling the effect of caffeine concentration to Caulobacter crescentus maximum specific growth rate based on statistical tests such as corrected AICc (Akaike Information Criterion), bias factor (BF), adjusted coefficient of determination (R2) and root-mean-square error (RMSE). Parameters obtained from the fitting exercise were maximum specific growth rate (q_{max}), K_s (concentration of substrate at the half maximal specific growth rate (mg/L) and K_i (inhibition constant) mg/L, with their values at 0.7620 (95%, C.I. or confidence interval from 0.603 to 0.920), 0.1050 mM (95% C.I., from 0.001 to 0.211) and 4.614 mM (95% C.I. from 2.154 to 7.073), respectively. These biologically meaningful coefficients will be useful in predicting growth conditions under batch studies and also probably under actual bioremediation strategy where the concentration of caffeine in the treatment may need be diluted to nontoxic concentration prior to remediation.

ACKNOWLEDGMENT

The authors would like to acknowledge Tertiary Education Trust Fund (Tetfund) and Bayero University, Kano for sponsorship and Universiti Putra Malaysia for providing an enabling environment to conduct the research.

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