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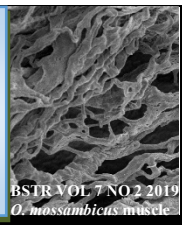

Modelling the Growth Inhibition Kinetics of *Rhodotorula* sp. strain MBH23 (KCTC 11960BP) on Acrylamide

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ABSTRACT

The yeast *Rhodotorula* sp. Strain MBH23 (KCTC 11960BP) is an efficient acrylamide-degrader and is able to tolerate high concentrations of acrylamide. A primary modelling exercise for the growth of this yeast on acrylamide yields important specific growth rates which were utilized successfully for secondary modelling exercise which gave Luong as the best model. The Luong's constants; maximal growth rate, half-saturation constant for maximal growth, maximal concentration of substrate tolerated 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 (± standard error) were 0.099±0.017 hr^{-1}, 17.34 ± 5.0 mg/L, 2053.0 ±56.0 mg/L and 0.801±0.202, respectively. The Luong model indicates that acrylamide is toxic and inhibits the growth of this yeast. To date, this is the first time that such a modelling exercise was utilized to model growth kinetics on acrylamide.

INTRODUCTION

Concern towards environmental problems is continuously growing due to the release of thousands of different toxic compounds as a result of human activities every day. Demands on the sustainable and controllable solutions to combat the environmental pollution which have minimal effect on the environment are highly sought after. Biodegradation is defined as a process of the disintegration of materials by the microorganism or other biological actions [1]. It is one of the established approaches for removal of undesirable organic compounds to the level that it can no longer be detected or within the maximum allowable limit set by the regulatory agencies [2].

Acrylamide (CH_2=CHCONH$_2$) is an amide group consisting of three-carbon compound with an α, β-unsaturated olefin bond. This compound is used as a commercial conjugated reactive molecule for the production of polymers especially polyacrylamide [3–5]. In industry, acrylamide has been used worldwide as a binding, thickening, and flocculating agent [6,7]. Acrylamide is also utilized in wastewater treatment method, pesticide ingredients, cosmetic makeup products, sugar production, and also to protect against soil deterioration. The prevalent utilization of acrylamide and its polymer (polyacrylamide) has resulted in the pollution of terrestrial and aquatic surroundings [3,4]. Acrylamide is a rising harmful pollutant. This poisonous substance could get into the body of a human by means of absorption by means of skin, lungs, and the digestive tract [8]. The exposure of human to acrylamide takes place mostly in place of work coming from skin touching the acrylamide monomer and from breathing in dust as well as vapor. Acrylamide is a known neurotoxicant, carcinogen and terratogen in mammals [6].

Acrylamide exerts its toxic effect when it is oxidized to the epoxide glycidamide that catalyzed by an enzymatic reaction involving cytochrome P450 2E1[9]. Previous studies showed that acrylamide and its oxidized form glycidamide caused abnormalities in the daughter cells of animals and plants [10]. In view of the fact that acrylamide is toxic to human health, this compound needs to be removed from the environment. Previously, several microorganisms such as *Pseudomonas* sp. [11], *Pseudomonas stutzeri* [12], *Pseudonocardia thermophila* [13], *Bacillus cereus* [14], and the fungi *Aspergillus oryzae* [15], which are capable of utilizing acrylamide a source of carbon and/or nitrogen have successfully been isolated. Moreover, our

group has also been successfully isolated and characterized an acrylamide-degrading yeast *Rhodotorula* sp. Strain MBH23 (KCTC 11960BP) [16].

Recently, a number of mathematical models have been utilized in order to explain the metabolism of compounds exposed to microbial populations in the natural environment. One of the most widely used mathematical equation in describing substrate utilization linked to growth rate is the Monod equation [17]. However, the limitation of this method is that it cannot be used for biodegradation process that shows substrate inhibition towards the rate. Due to this limitation, a model such as the Haldane or other substrate-inhibiting models such as Aiba, Webb (Edward), Teissier Yano and Koga, Hans-Levenspiel and Luong have been preferably used [18,19]. Hence, the utilization of considerable models available could replace the Haldane in some circumstances and discloses mechanistic process.
In the present study, the growth of strain MBH23 (KCTC 11960BP) was inhibited when the acrylamide concentration in the growth media was increased to 2000 mg/L.

The inhibition of the bacterial growth rate caused by the high phenol concentration has not been seen in many phenol biodegradation studies using *Pseudomonas* sp. bacteria model, where the Haldane and other kinetics model have been used instead of models that allow for the complete abolishment of growth rates such as Luong, Teissier and Han-Levenspiel. This is probably due to the well-perceived notion that the *Pseudomonas* genus is highly tolerant to toxicants including phenol [20–34]. In order to obtain more accurate data, the bacteria growth rates on phenol were determined by employing the modified Gompertz model as a nonlinear curve fitting model [28,35–42]. In many previous phenol biodegradation studies, the growth rates were obtained by taking the linear portion of the natural logarithm of cellular biomass, which is done manually. Using the growth rates data, several available growth kinetic models were then evaluated. To date, limited statistical tests were used to accept the best model in modelling the kinetics of phenol biodegradation, and the most commonly used test is the coefficient of determination (R^2) [43,44].

However, by using this coefficient of determination (R^2), the number of parameters used in the model needs to be adjusted [45–47]. This adjustment can be made using an adjusted coefficient of determination (adjR^2), root mean square error RMSE, Corrected Akaike Information Criteria (AICc) and others. Based on the data obtained in this study, Luong model was found to be the best model to study the phenol biodegradation by the bacteria. In addition, the use of nonlinear regression fitting and assessment of the best model based on statistical tests relies heavily on the premise that the residuals are normally distributed. Thus, three commonly used statistical diagnosis tests for normality which are Wilks-Shapiro, Kolmogorov-Smirnov, and D'Agostino-Pearson were performed on the residuals from the Luong model. This present study suggests that in order to obtain an accurate and more reliable data, future selection of the best kinetic model governing the growth rates on toxicants especially acrylamide should be done comprehensively.

MATERIALS AND METHODS

Growth and maintenance of acrylamide-degrading yeast

The yeast *Rhodotorula* sp. Strain MBH23 (KCTC 11960BP) was maintained in minimal salts medium (MSM). The MSM (pH 7.5) with glucose autoclaved separately is composed of (per liter): 6.8 g of KH_2PO_4 (BDH),10 g of glucose (BDH (British Drug House), Poole, UK), 0.005 g of $FeSO_4 \cdot H_2O$ (BDH), 0.5 g of $MgSO_4 \cdot 7H_2O$ (BDH), 0.5 g acrylamide as the sole nitrogen source with 1 mL of the following trace elements (per liter): 0.003 g of $CoCl_2 \cdot 6H_2O$, 0.01 g of $Cu(CH_3COO)_2.H_2O$ 0.03 g of $ZnCl_2$ (BDH); 0.002 g of $FeCl_2 \cdot 6H_2O$ (JT Baker) and 0.05 g of H_3BO_3 (JT Baker, John Townsend Baker, Phillipsburg, N.J., U.S.A.) [48]. In order to avoid degradation via heating, acrylamide was sterilized by passing through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter. The culture was incubated on a shaking incubator (Certomat R, USA) at room temperature (28 °C) at 150 rpm for 72 h [16].

Growth kinetics on acrylamide

The yeast growth kinetics on acrylamide was studied using a batch culture of the yeast supplemented with acrylamide at concentrations up to 1000 mg/L. The modified Gompertz model was utilised in the secondary inhibition kinetics modelling to obtain the growth parameter maximum specific growth rate or μ_m. The equation is as follows;

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\} \quad (1)$$

The values obtained from this primary modelling exercise was then used to model various growth kinetics model as follows;

Table 1. Kinetic models for growth of yeast on acrylamide.

Author	Degradation Rate	Ref
Monod	$\mu_{\max}\frac{S}{K_S+S}$	[17]
Haldane	$\mu_{\max}\frac{S}{S+K_S+\frac{S^2}{K_i}}$	[49]
Teissier	$\mu_{\max}\left(1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_S}\right)\right)$	[50,51]
Aiba	$\mu_{\max}\frac{S}{K_S+S}\exp(-KP)$	[52]
Yano and Koga	$\frac{\mu_{\max}S}{S+K_S+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[53]
Luong	$\mu_{\max}\frac{S}{S+K_S}\left[1-\left(\frac{S}{S_m}\right)^n\right]$	[54]

Note:
μ_{max} maximal growth rate (h^{-1})
K_s half saturation constant for maximal degradation (mg/L)
S_m maximal concentration of substrate tolerated and (mg/L)
m, n, K curve parameters
S substrate concentration (mg/L)
P product concentration (mg/L)

Fitting of the data

Nonlinear regression was carried out using the CurveExpert Professional software (Version 1.6), which utilizes the Marquardt algorithm to fit the Gompertz and several inhibition kinetics models (**Table 1**) by nonlinear regression. This algorithm reduces the sums of squares of the residuals.

Statistics of the growth kinetics

Statistical analysis of the growth models' residuals was carried out to select the best model, using approaches such as the corrected Akaike Information Criterion or AICc, adjusted coefficient of determination (R^2), root-mean-square error (RMSE) accuracy factor (AF) and bias factor (BF). Statistical diagnosis tests for normality which are Wilks-Shapiro, Kolmogorov-Smirnov, and D'Agostino-Pearson were performed on the residuals from the Luong model [55].

The RMSE was calculated according to equation 2,

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

where

n number of experimental data
Pd_i predicted values by the model
Ob_i experimental data
p parameters number of the model

In general, the model having the smaller number of parameter results in a smaller RMSE value [56].

The coefficient of determination or R^2 although popular the method does not consider the number of parameters of models in nonlinear regression, and therefore does not readily offer comparative evaluation. To get over this problem, an adjusted R^2 which takes into consideration the quantity of parameter of models is utilized to calculate the quality of nonlinear models based on the formula below;

$$Adjusted\ (R^2) = 1 - \frac{RMS}{s_Y^2} \quad (3)$$

$$Adjusted\ (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \quad (4)$$

where

s_y^2 is the total variance of the y-variable and RMS is the Residual Mean Square

The Akaike information criterion (AIC) is established upon information theory. The formula incorporates some variables penalty where the more the variables, the higher the AIC value. In studies where the data is small a corrected version of AIC; the Akaike information requirements (AIC) with correction or AICc is utilised instead [57]. AICc is calculated using the following equation;

$$AICc = 2p + n\ln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad (5)$$

Where

n number of data points
p parameter numbers of the model

The Accuracy Factor (AF) and Bias Factor (BF) are another goodness-of-fit exercises for models (Ross and McMeekin, 1994). The statistics calculates the perfect match between experimental and predicted values. As a rule, a BF value > 1.0 indicates a model which is fail-safe a value < 1.0 indicates a model that is fail-dangerous. On the other hand, the AF is always ≥ 1.0, with precise models giving values nearing to 1.0.

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \quad (6)$$

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

RESULTS AND DISCUSSION

Growth kinetics

The specific growth rate was obtained from a primary modelling exercise using the modified Gompertz model (**Fig. 1**) as this model has often been used to model growth curve on xenobiotics as a substrate [58,59].

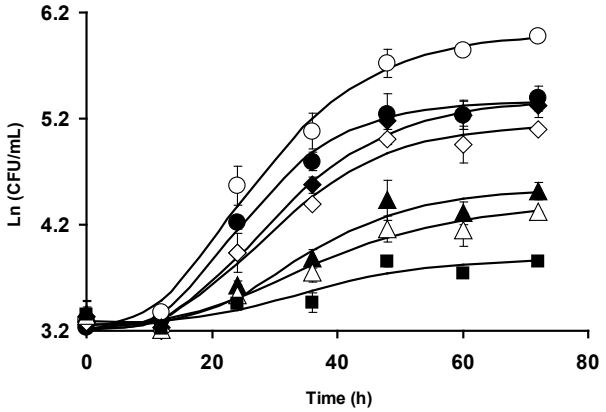

Fig. 1. Growth of *Rhodotorula* sp. Strain MBH23 on various acrylamide concentrations (200 (●), 300 (○), 400 (◆), 500 (◇), 600 (▲), 700 (△) and 800 (■) mg/L) modelled according to the modified Gompertz model (line). Error bars indicate mean standard deviation (n=3).

The primary modelling exercise yields important specific growth rates which were then plotted against the initial acrylamide concentrations. A secondary modelling exercise utilizing various kinetics models was then carried out (**Figs. 2-7**). The statistical analysis and accuracy of the all six kinetic models used indicated that Luong was the best model with small values for RMSE and AICc, uppermost adjusted R^2 values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0) (**Table 2**).

The Luong's constants; maximal growth rate, half-saturation constant for maximal growth, maximal concentration of substrate tolerated 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 (± standard error) were 0.099±0.017 hr^{-1}, 17.34 ± 5.0 mg/L, 2053.0 ±56.0 mg/L and 0.801±0.202, respectively. 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 failed to explain some situations where growth rate became zero at very high substrate concentration

[60]. To date, this is the first time that such a modelling exercise was utilized to model growth kinetics on acrylamide. Modelling of bacterial growth kinetics on xenobiotics is an important aspect of designing effective bioremediation strategy as the constants obtained can be utilized to plan and understand the limitations of bioremediation [19].

Table 2. Statistical analysis of kinetic models.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Luong	4	0.011	1.00	0.99	-59.11	1.00	1.03
Aiba	3	0.013	0.96	0.94	-47.19	0.98	1.04
Haldane	3	0.011	0.97	0.96	45.45	1.00	1.15
Han and Levenspiel	4	0.025	0.87	0.75	-33.12	1.00	1.15
Yano	4	0.025	0.87	0.75	-35.16	1.00	1.15
Teissier	4	0.025	0.89	0.79	-33.44	0.89	1.22
Monod	2	0.063	-4.09	-5.78	-36.17	0.89	1.54

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

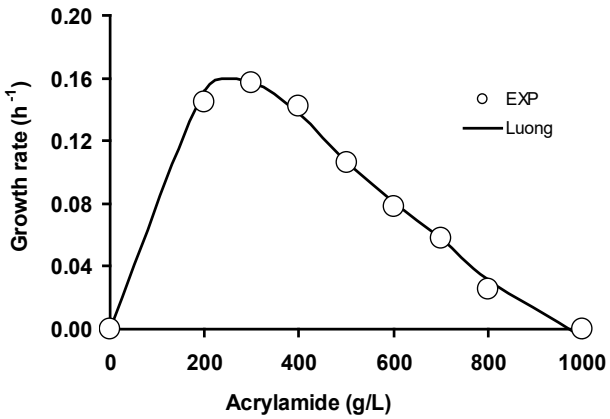

Fig. 3. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Luongs's model.

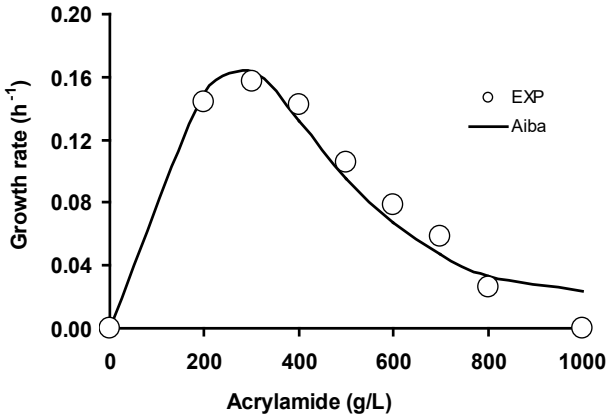

Fig. 2. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Aiba's model.

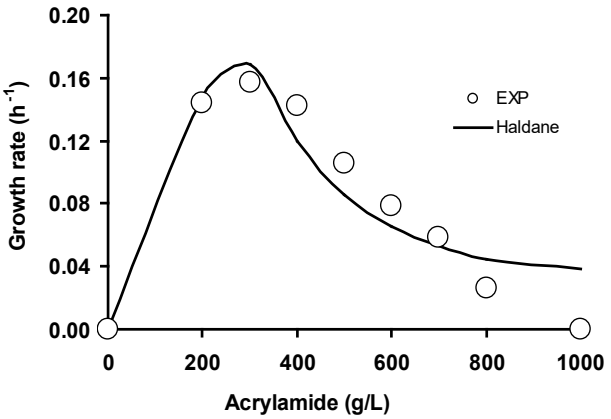

Fig. 3. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Haldane's model.

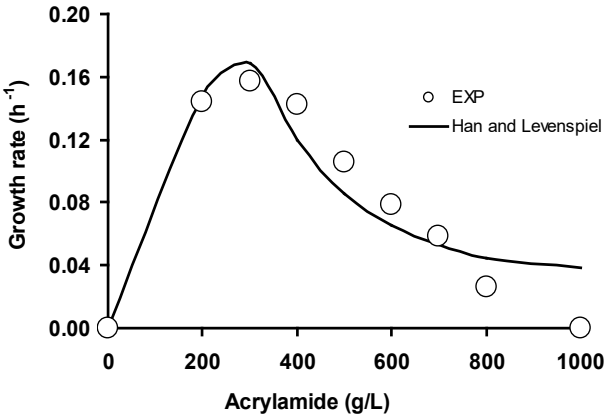

Fig. 4. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Hans and Levenspiel's model.

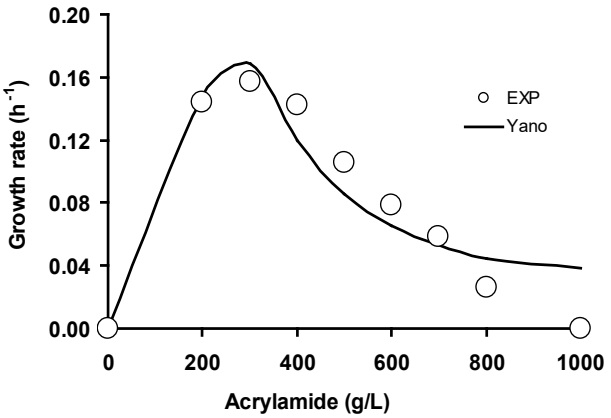

Fig. 5. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Yano's model.

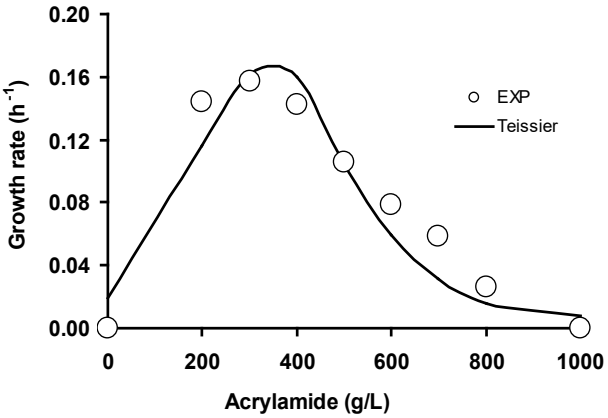

Fig. 6. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Teissier's model.

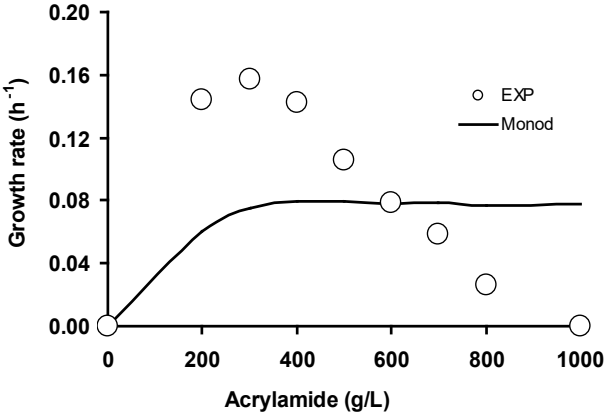

Fig. 7. Curve fitting of the growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide using the Monod's model.

CONCLUSION

In conclusion, the primary modelling exercise for the growth of this yeast on acrylamide yields important specific growth rates which were utilized successfully for secondary modelling exercise which gave Luong as the best model. The Luong's constants; maximal growth rate, half-saturation constant for maximal growth, maximal concentration of substrate tolerated 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 (± standard error) were 0.099±0.017 hr^{-1}, 17.34 ± 5.0 mg/L, 2053.0 ±56.0 mg/L and 0.801±0.202, respectively. The Luong model indicates that acrylamide is toxic and inhibits the growth of this yeast. To date, this is the first time that such a modelling exercise was utilized to model growth kinetics on acrylamide.

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