

## Modelling the Effect of Heavy Metals on the Growth Rate of *Enterobacter* sp. Strain Neni-13 on SDS

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mercury  
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### ABSTRACT

The SDS-degrading bacterium *Enterobacter* sp. strain Neni-13 was strongly inhibited by mercury. Growth of the SDS-degrading bacterium at various concentrations of mercury shows a sigmoidal pattern with lag periods ranging from 7 to 10 h. As the concentration of mercury was increased, the overall growth was inhibited with 1.0 mg/L causing an almost cessation of bacterial growth. The growth rates obtained from the modified Gompertz model was then modelled according to the modified Han-Levenspiel, Wang, Liu, modified Andrews and the Amor models. Out of the five models, only Wang, modified Han-Levenspiel and the Liu models were able to fit the curve, whilst the modified Andrews and Amor models were unable to fit the curves. Both the Wang and modified Han-Levenspiel models show acceptable fitting while the Liu model shows poor fitting. Results of the statistical analysis showed that the Wang model was the best model based on the lowest values for RMSE and AICc, highest adjusted correlation coefficient ( $adjR^2$ ) and values of AF and BF closest to unity. The parameters obtained from the Wang model, which are  $C_{crit}$ ,  $\mu_{max}$  and  $m$  which represent critical heavy metal ion concentration (g/l), maximum growth rate (g/l h) and empirical constant values were 0.216 (95%, confidence interval f 0.193 to 0.239), 1.05 (95%, confidence interval of 0.938 to 1.167) and 0.389 (95%, confidence interval of 0.148 to 0.636) respectively.

### INTRODUCTION

The existence of toxic metal ions in polluted comprising wastewater displayed an inhibition influence on the bacterial growth and utilization of toxic substance. The presence of heavy metals can inhibit biodegradation and ultimately inhibit bioremediation process. It is because of the fact that in contrast to a number of other inhibitors, heavy metal ions cannot be degraded and once accrued by microorganisms to a poisonous amount, this result in an inhibition to the microorganism's growth rate. Therefore, modifications to the substrate inhibition model can be used to examine the inhibitory parameters caused by toxic ions. Numerous models such as the modified Han-Levenspiel [1], Wang [2], Liu [3], modified Andrews[4], Amor [5] have been utilised [6] to evaluate the result of heavy metal on the bacterial degradation of toxic substance. From these models inhibition related constants, which include  $C$ ,  $C_{crit}$ ,  $\mu$ ,  $\mu_{max}$ ,  $K_c$ ,

$K_s$ ,  $K_i$  and  $m$  which represent heavy metal ion concentration (g/l), critical heavy metal ion concentration (g/l), initial growth rate (g/l h), maximum growth rate (g/l h), inhibition constant (g/l), Monod constant (g/l), metal inhibition constant (g/l) and empirical constant values, respectively, can be found.

To date aside from these reports, there are almost no other reports on the effect of heavy metals on the growth rate of microorganisms as most reports on the effect of heavy metals on the primary models of the growth of microorganisms and not on secondary models. A previously isolated SDS-degrading bacterium was shown to be strongly inhibited by the heavy metals mercury, silver and copper [7,8]. The aim of this work is to study the effect of mercury on the growth rate of this bacterium on SDS through the use of several inhibition models.

**Growth and maintenance of SDS-degrading bacterium**

The SDS-degrading bacterium—*Enterobacter* sp. strain Neni-13 has been previously reported [7,8]. The growth of the bacterium on SDS was characterized in a microtiter plate format [9,10]. The bacterium was grown on a basal salts (BS) medium containing the followings: Na<sub>2</sub>HPO<sub>4</sub>, (1.39 g l<sup>-1</sup>), KH<sub>2</sub>PO<sub>4</sub>, (1.36 g l<sup>-1</sup>), KNO<sub>3</sub>, (0.5 g l<sup>-1</sup>), CaCl<sub>2</sub> (0.01 g l<sup>-1</sup>), MgSO<sub>4</sub> (0.01 g l<sup>-1</sup>), and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (7.7 g l<sup>-1</sup>) and 1 mL of trace elements [7]. SDS was added into the medium (filter-sterilized) at 1.0 g l<sup>-1</sup>. The microplates (Corning® microplate) were incubated sealed at 30 °C and was read at 600 nm (BioRad reader, model 680, Richmond, CA).

**Primary growth modelling on SDS**

The maximum specific growth rate on SDS was modelled according to the modified Gompertz model as this model is routinely used in modelling the growth of microorganisms on xenobiotics [11–13]. The equation (Eqn. 1) is as follows;

$$y = A \exp \left\{ - \exp \left[ \frac{\mu_{me} (\lambda - t)}{A} + 1 \right] \right\} \quad (\text{Eqn. 1})$$

The value obtained from this primary modelling exercise was then used to model the effect of metal as follows;

**Effect of metal on growth rate of on SDS**

The models utilized in this study is as follows (Table 1);

**Table 1.** Metal inhibition models.

Models	Equation	Ref
Modified Han-Levenspiel	$r = u_{max} \left( 1 - \frac{C}{C_{crit}} \right)^m$	[1]
Wang	$r = \frac{u_{max}}{1 + \left( \frac{C}{K_C} \right)^m}$	[2]
Liu	$r = \frac{u_{max} K_C}{K_C + C}$	[3]
Modified Andrews	$r = \frac{u_{max} C}{K_S + C + \left( \frac{C^2}{K_i} \right)}$	[4]
Amor	$r = \frac{u_{max} C}{C + \left( \frac{C^2}{K_i} \right)}$	[5]

**Fitting of the data**

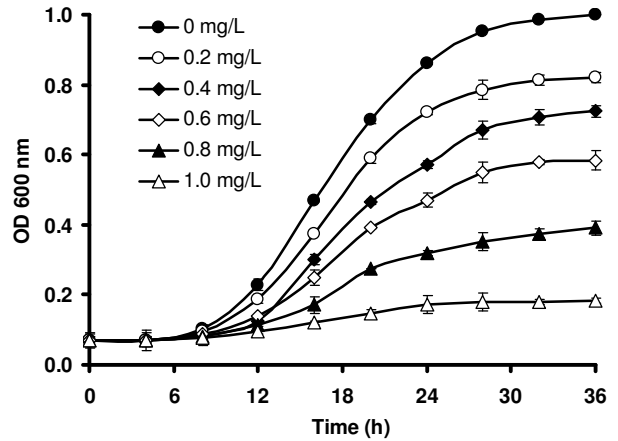
The nonlinear equations were fitted with a Marquardt algorithm using CurveExpert Professional software (Version 1.6). The algorithm searches the best method that minimizes the sum of the squares between predicted and measured values. The software calculates the starting values automatically through via the steepest ascent method.

**Statistical analysis**

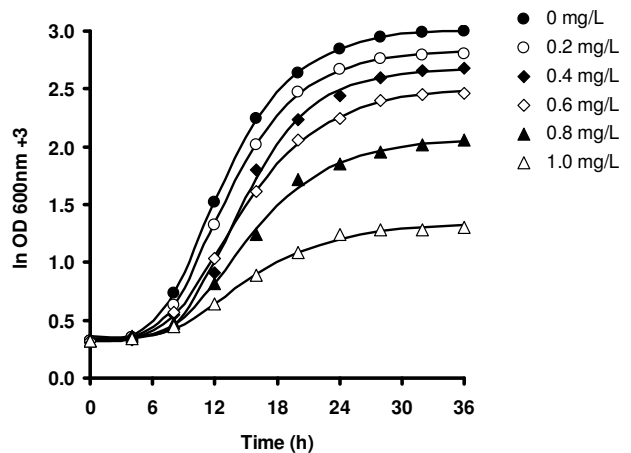
To choose the best model, numerous statistical methods including the corrected AICc (Akaike Information Criterion), Root-Mean-Square Error (RMSE), bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (R<sup>2</sup>) was utilized as before [14].

**RESULTS AND DISCUSSION**

Growth of the bacterium at various concentrations of mercury shows a sigmoidal pattern with lag periods ranging from 7 to 10 h (Fig. 1). As the concentration of mercury was increased, the overall growth was inhibited with 1.0 mg/L causing an almost cessation of growth. To obtain growth rates at different concentrations of mercury, the modified Gompertz model was utilized (Fig. 2), which shows close fitting to the model. The model also shows that as the concentration of mercury was increased, this led to a decrease in growth rates and an increase in lag period as well.



**Fig. 1.** Growth of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L). The error bars represent mean ± standard deviation of triplicates.



**Fig. 2.** Growth (log transformed) of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L) as modelled using the modified Gompertz model.

The growth rates at various concentrations of mercury was then modelled using the available metal inhibition models. Out of the five models, only Wang, modified Han-Levenspiel and the Liu models were able to fit the curve, whilst the modified Andrews and Amor models were unable to fit the curves (Figs. 3 to 5). Both the Wang and modified Han-Levenspiel models show acceptable fitting while the Liu model shows poor fitting. Results of the statistical analysis showed that the Wang model was the best model based on the lowest values for RMSE and AICc,

highest adjusted correlation coefficient ( $adR^2$ ) and values of AF and BF closest to unity (Table 2).

Table 2. Statistical analysis of various metal inhibition models.

Model	$p$	RMSE	$R^2$	$adR^2$	AF	BF	AICc
Wang	3	0.00	0.99	0.99	1.02	1.01	-44.57
Modified Han-Levenspiel	3	0.01	0.98	0.97	1.03	1.00	-37.51
Liu	2	0.04	0.22	0.17	1.20	1.02	-30.58
Modified Andrews	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Amor	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Note:

$p$  no of parameter

$adR^2$  adjusted correlation coefficient

RMSE Root mean square error

AF Accuracy factor

BF Bias factor

AICc corrected Akaike Information Criteria

n.a. not available

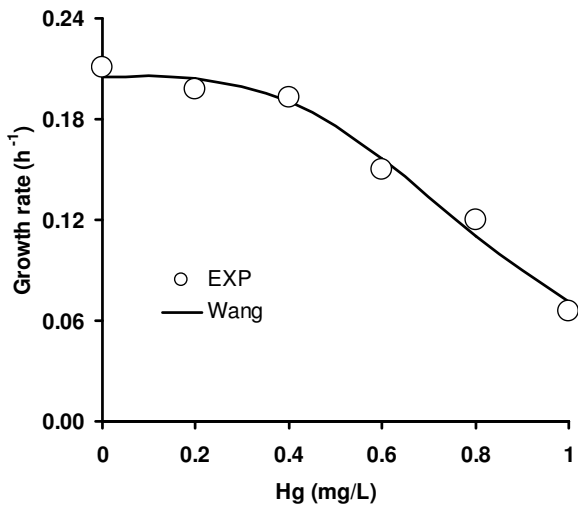


Fig. 3. Growth rate of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L) as modelled using the Wang model.

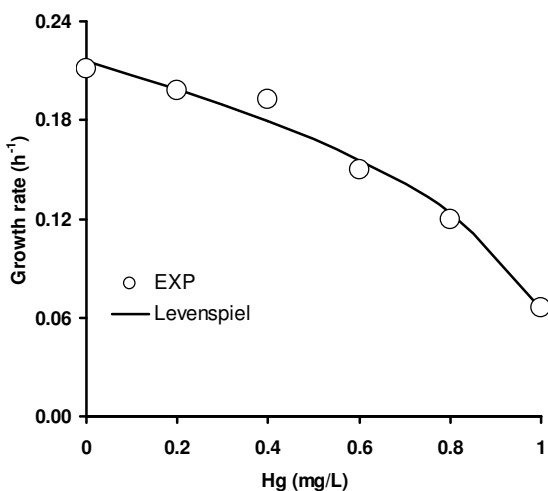


Fig. 4. Growth rate of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L) as modelled using the Han-Levenspiel model.

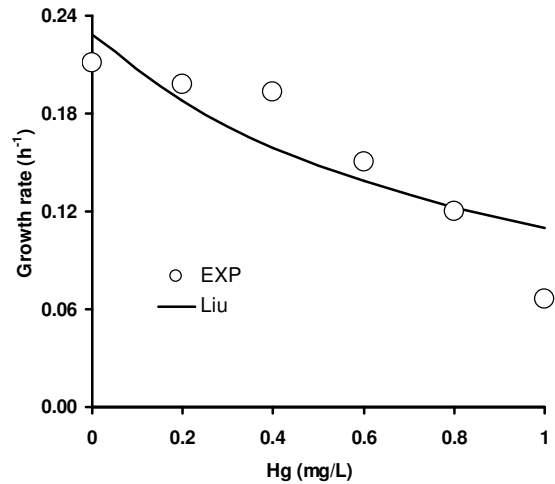


Fig. 5. Growth rate of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L) as modelled using the Liu model.

The parameters obtained from the Wang model, which are  $C_{crit}$ ,  $\mu_{max}$  and  $m$  which represent critical heavy metal ion concentration (g/l), maximum growth rate (g/h) and empirical constant values were 0.216 (95%, confidence interval of 0.193 to 0.239), 1.05 (95%, confidence interval of 0.938 to 1.167) and 0.389 (95%, confidence interval of 0.148 to 0.636) respectively. The Wang model allows for the prediction of the critical heavy metals concentration which can completely inhibited bacterial growth.

The use of metal inhibition models is poorly represented in the literature despite the importance of such study in light of the fact that heavy metals are ubiquitously present in polluted waters alongside organic pollutants. A few studies have explored on the effect of heavy metals of the growth rate of bacteria growing on toxic substance. For example, the biodegradation rate of monoaromatic hydrocarbons by *Bacillus* sp. and *Pseudomonas* sp. was inhibited strongly by zinc and nickel and the effect of these heavy metals on the degradation rate was successfully modelled by using the Andrews model [5]. Heavy metals bind to important functional groups of enzymes such as the sulfhydryl group that are often found at the active sites of enzymes and this is probably the mechanism of inhibition [6].

## CONCLUSION

In conclusion, the use of metal inhibition models to model the effect of metal ions to the growth rate of bacteria on toxic substances is very rare and largely ignored despite the importance of such study. In this study the effect of mercury to the growth of an SDS-degrading bacterium on SDS was modelled according to several metal inhibition models, with the Wang model discovered as the best model. The Wang model allows for the prediction of the critical heavy metals concentration which can completely inhibited bacterial growth. It is expected that in the presence of heavy metals, the growth rate on toxic substance will be even strongly affected as the bacteria have to resist the toxicity of both kind of toxicants at the same time. The results from this study can be very important in field trial works where SDS bioremediation is sought in areas co-contaminated with mercury.

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