Modelling the Inhibitory Effect of Mercury on the Growth Rate of a Bacterium on Acrylamide

Motharasan Manogaran1,2*, Syahir Habib1,3, Mohd Badrin Hanizam Abdul Rahim1,3, Aisami Abubakar4, Umar Abubakar Muhammad5 and Ibrahim A. Allamin6

1Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43000 Kajang, Selangor, Malaysia.
2Malaysia Genome and Vaccine Institute (MGVI) National Institute of Biotechnology Malaysia (NIBM) Jalan Bangi, 43000 Kajang, Selangor, Malaysia.
3Agribiotechnology Group, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
4Department of Biochemistry, Faculty of Science, Gombe State University, P.M.B 127, Tudun Wada, Gombe, Gombe State, Nigeria.
5Department of Biological Sciences, Faculty of Science, Gombe State University, P.M.B 127, Tudun Wada, Gombe, Gombe State, Nigeria.
6Department of Microbiology, Faculty of Science, University of Maiduguri P.M.B 1069 Maiduguri, Nigeria.

*Corresponding author:
Dr. Motharasan Manogaran,
Malaysia Genome and Vaccine Institute (MGVI),
National Institute of Biotechnology Malaysia (NIBM),
Jalan Bangi,
43000 Kajang,
Selangor,
Malaysia.
Email: motharasan@gmail.com

ABSTRACT

Mercury was a potent inhibitor of the acrylamide-degrading Pseudomonas sp. strain DrY Kertih bacterium. The bacterium is capable of growing on acrylamide as a nitrogen source. Growth shows a sigmoidal pattern with lag times of 7–10 hours when exposed to increasing amounts of mercury. Bacterial growth was significantly slowed down while the lag period was increased as the concentration of mercury was increased to 0.1 mg/L, and eventually, growth stopped altogether. Growth rates at various mercury concentrations were calculated using a modified Gompertz model to obtain the maximum specific growth rate parameter. The modified Han-Levenspiel, modified Andrews, Shukor, Kai, Liu, Wang and Amor models were applied to the growth rates. The Amor model was unable to fit the curve. The Wang model performed the best statistically, with the lowest RMSE and AICc, the highest adjusted correlation coefficient (adR2), and AF and BF values closest to unity. The parameters obtained were \( K_c \), \( \mu_{max} \) and \( m \) which represent critical heavy metal ion concentration (g/l), and maximum growth rate (per h) and empirical constant values were 0.054, 0.431 and 4.295, respectively. The results obtained in this study are useful when remediation works on polluted sites containing acrylamide co-contaminated with metal ions especially mercury.

INTRODUCTION

Acrylamide-induced seminiferous tubule histological abnormalities damage male rats' reproductive systems. Chemicals produce histological abnormalities. If inhaled or ingested, acrylamide may produce burning or a rash. Overactive sweating, sluggishness, and tongue trembling indicate neurological issues [1]. Acrylamide binds to DNA and mouse protamine during the spermatogenic process in mice, causing genetic diseases [2]. Research has connected acrylamide exposure in rats to endocrine-related malignancies, perinatal mortality, clastogenicity, mutagenicity, and male reproductive toxicity [3]. Spencer and Schaumburg found that acrylamide exposure in experimental animals caused cancer, but it is unclear if the same is true for humans [1]. Yang et al. found that acrylamide may mutate Salmonella strains TA100 and TA98. Mice injected intraperitoneally with 50 mg/kg acrylamide showed more chromosomal abnormalities in their bone marrow [4]. Acrylamide intraperitoneal doses up to 125 mg/kg did not increase chromosomal abnormalities in mice lymphocytes.
Intraperitoneal acrylamide was found to cause this [5]. Acrylamide can be absorbed through the lungs, skin, placental barrier and the digestive system due to its high water solubility. Acrylamide adducts in haemoglobin can be used to estimate the public's exposure to acrylamide due to their profession. According to the data, 41 acrylamide industrial workers had haemoglobin adduct-related neurotoxicity. Haemoglobin adducts surged in workers from a Chinese acrylamide plant, indicating that they were exposed to exceptionally high amounts of acrylamide [6]. Japanese water contamination cases have caused many cases of acute acrylamide poisoning. Multiple individuals have experienced this [7].

Acrylamide, a neurotoxic and carcinogenic chemical, can develop during the Maillard reaction. Carbohydrate-heavy foods cooked at high temperatures can produce acrylamide. Acrylamide is produced by the Maillard process in carbohydrate-rich meals. The Maillard process begins when carbohydrates in food and amino acids are mixed at a very high temperature especially frying [8]. Acrylamide-contaminated streams in Sweden and Norway killed cattle and fish. Polyacrylamide (PAM) is the most prevalent use for acrylamide in the production of adhesives, plastics, printed goods, and drinking water. As of 2005, commercial polyacrylamides often contain the deadly mercury on the growth curve of the bacterium on acrylamide as the nitrogen source was carried out by adding mercury (Merck Atomic Absorption standard) from 0.01 to 0.1 mg/L final concentration.

Modified Gompertz model as the primary model in modelling growth on acrylamide

The modified Gompertz model is a predominantly fast convergence model that is commonly used to fit bacteria growth on toxic xenobiotics [28–32]. The prerequisite of using this model and other primary models to obtain the maximum specific growth rate is the values of growth, either in dry weight, A600nm, OD 600nm or CFU/mL have to be ln transformed [33–35], as the growth rate unit is per hour or per d. The equation is as follows;

\[ y = \exp \left\{ \frac{m_m}{a} \left(1 - \exp \left(\frac{-k_a x}{a}\right)\right) \right\} \]

**Inhibitory models for growth rate on acrylamide**
The result of this preliminary modelling effort was utilized to model (Table 1) the influence of metal in the following models;

<table>
<thead>
<tr>
<th>Models</th>
<th>Equation</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified Han-</td>
<td>[ r = \frac{u_{max}}{1 + \frac{C}{K_u}} ]</td>
<td>[10]</td>
</tr>
<tr>
<td>Levenspiel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wang</td>
<td>[ r = \frac{u_{max}}{1 + \frac{C}{K_u}} ]</td>
<td>[12]</td>
</tr>
<tr>
<td>Liu</td>
<td>[ r = \frac{u_{max}K_C}{K_C + C} ]</td>
<td>[14]</td>
</tr>
<tr>
<td>Modified Andrews</td>
<td>[ r = \frac{K_0 + C}{K_0 + C + \left(\frac{1}{X}\right)^n} ]</td>
<td>[13]</td>
</tr>
<tr>
<td>Amor</td>
<td>[ r = \frac{u_{max}K_C}{K_C + C} ]</td>
<td>[17]</td>
</tr>
<tr>
<td>Shukor</td>
<td>[ r = \frac{u_{max}}{1 - \left(\frac{C}{K_u}\right)} ]</td>
<td>[11]</td>
</tr>
<tr>
<td>Kai</td>
<td>[ r = u_{max}\exp \left(\frac{-k_a x}{a}\right) ]</td>
<td>[15]</td>
</tr>
</tbody>
</table>

**Growth Fitting**
CurveExpert Professional software was used in the modelling exercise. The software applies a Marquardt algorithm to the nonlinear equations (Version 1.6). To find the optimal solution, the algorithm looks for the smallest possible squared difference between the anticipated and actual numbers. The steepest ascent approach is used automatically by the software to determine the initial values.

**Statistical analysis**
To choose the best model, numerous statistical methods or error function analyses were utilized such as Ross’s bias factor (BF) and accuracy factor (AF) [36], which is a non-penalty method for the number of the parameters of models and penalty-imposing methods to the number of parameters of models such as corrected adjusted coefficient of determination (R²), AICc (Akaike Information Criterion) and Root-Mean-Square Error (RMSE) [37].

**MATERIALS AND METHODS**

**Growth and maintenance of acrylamide-degrading bacterium**
The acrylamide-degrading bacterium—*Pseudomonas* sp. strain DrY Kertih has been previously isolated in our lab [24]. Characterization of bacterial growth on acrylamide was performed using microtiter plates (Corning® microplate) [25,26]. The bacterium was grown on glucose as a sole source of carbon and in a basal salts (BS) medium containing the following; KH₂PO₄, (1.36 g l⁻¹), Na₂HPO₄, (1.39 g l⁻¹), CaCl₂: (0.01 g l⁻¹), MgSO₄: (0.01 g l⁻¹), and glucose (10 g l⁻¹) [27]. The final volume was 0.2 mL. Acrylamide was filter-sterilized (0.2-µm filter membrane Teflon) to a final concentration of 1.0 g L⁻¹. The microplates were incubated sealed at 30°C resulting in static growth and the increase in turbidity due to bacterial growth was read at 600 nm (BioRad reader, model 680, Richmond, CA). The control contained everything minus acrylamide. The effect of mercury on the growth curve of the bacterium on acrylamide as the nitrogen source was carried out by adding mercury (Merck Atomic Absorption standard) from 0.01 to 0.1 mg/L final concentration.

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RESULTS AND DISCUSSION

The bacterial growth curves have a sigmoidal shape at different mercury concentrations, with lag times between 7 and 9 hours (Fig. 1). Overall growth was decreased as mercury content was increased, with 0.1 mg/L practically completely interrupting bacterial growth. Curve fitting of ln transformed bacterial growth at various mercury concentrations utilizing the modified Gompertz model (Fig. 2) indicates a high degree of model fit with values of coefficient of determination or $R^2$ >0.95 for each curve. The model also demonstrates that an increase in mercury levels resulted in slower growth development rates and a lengthier lag time at all concentrations was observed.

Acceptable fitting is seen in the Wang, Shukor and modified Han-Levenspiel models, but poor fitting for the rest of the models. Statistical analysis showed that the Wang model was the best model. This is based on RMSE and AICc’s lowest values, the highest adjusted correlation coefficient ($adR^2$) and the closest to unity for AF and BF (Table 2).

Table 2. Error function analysis for all models.

<table>
<thead>
<tr>
<th>Model</th>
<th>p</th>
<th>RMSE</th>
<th>$R^2$</th>
<th>$adR^2$</th>
<th>AF</th>
<th>BF</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang</td>
<td>3</td>
<td>0.01</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>1.00</td>
<td>-28.91</td>
</tr>
<tr>
<td>Modified Hans-Levenspiel</td>
<td>3</td>
<td>0.04</td>
<td>0.94</td>
<td>0.88</td>
<td>1.07</td>
<td>1.01</td>
<td>-13.96</td>
</tr>
<tr>
<td>Liu</td>
<td>2</td>
<td>0.09</td>
<td>0.27</td>
<td>-0.09</td>
<td>1.12</td>
<td>0.96</td>
<td>-17.62</td>
</tr>
<tr>
<td>Modified Andrews</td>
<td>3</td>
<td>0.06</td>
<td>0.86</td>
<td>0.72</td>
<td>2.46</td>
<td>0.41</td>
<td>-10.18</td>
</tr>
<tr>
<td>Shukor</td>
<td>3</td>
<td>0.04</td>
<td>0.96</td>
<td>0.92</td>
<td>1.08</td>
<td>1.01</td>
<td>-16.77</td>
</tr>
<tr>
<td>Kai</td>
<td>2</td>
<td>0.07</td>
<td>0.72</td>
<td>0.58</td>
<td>1.10</td>
<td>0.97</td>
<td>-21.91</td>
</tr>
</tbody>
</table>

Note: $adR^2$ adjusted correlation coefficient
RMSE Root mean square error
AICc corrected Akaike Information Criteria
AF Accuracy factor
BF Bias factor
n.a. not available
p no of parameter

The growth rates data at a range of mercury concentrations was then modelled using the Wang, Shukor, modified Han-Levenspiel, and modified Andrew, Kai and Liu models. The Amor model was unable to fit the data (Figs. 3 to 8).
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The inhibitory effects of metal ion on the growth or degradation rate of microorganisms is poorly modelled despite the importance of the outcome in identifying whether a certain inhibitor either partially or completely inhibited rates of growth or degradation. For instance, zinc and nickel ions strongly hindered the rate of the biodegradation of the xenobiotics monoaromatic hydrocarbons by two bacteria; Bacillus sp. and Pseudomonas sp.

The best model governing the inhibitory kinetics is the Andrews model which efficiently predicts the inhibitory influence of these ions on the rate of degradation [17]. To date, one of the most popular models utilized in modelling the effect of inhibitors be them metal ions, organic or inorganic on the growth of compounds, production of metabolites or degradation rate of pollutants is the modified Han-levenspiel model where its application has been reported [16,38–42].

Heavy metals bind to critical functional groups impairing the enzyme activity. A critical functional group such as the sulphydryl group is most easily bound [18]. When metal ions bind to the sulphydryl (-SH) groups in active sites of enzymes necessary for microbial metabolic reactions, poisoning of the metabolic pathway results. In fact, the dissociation constant of a metal sulfide has been found to be inversely proportional to the minimum inhibitory concentration or MIC of that metal against Escherichia coli (Nies 1999). Metal ions may impede the degradation of pollutants or the production of industrially useful products such as hydrogen or methane by interfering with enzymes including dehydrogenases and oxygenases, which are broadly involved in metabolisms of xenobiotics. There are several strategies for allowing the biodegradation process to proceed in the occurrence of heavy metal ions that impede the process.

A metal-resistant bacterium’s presence as a co-degrader can accelerate the breakdown process when paired with a main bacterial degrader. In one study, 2,4-D-soil polluted with cadmium was inoculated with the cadmium-resistant bacterium Pseudomonas H1, which efficiently sequestered cadmium in the cytoplasm, and a 2,4-D-degrading bacterium added to the targeted toxicant allows biodegradation process to proceed in soil microcosms studies. According to the study, inoculating a system with a cadmium-resistant bacterium allows the cadmium concentration in the soil to be not bioavailable for inhibiting xenobiotics-degrading bacterium in the neighbouring environment, promotes the biodegradation of the targeted organic contaminant [43].

Clay minerals are another option. These minerals are able to sorb heavy metal ions and have been used to decrease the bioavailability of metals and, thus, their toxicity. Clay minerals absorb heavy metals by a number of intricate adsorption mechanisms, including surface complexation, direct bonding of metals to the surface of the clay mineral, and ion exchange. For instance, the toxicity of cadmium was mitigated for bacteria, yeasts, and an actinomycete when kaolinite (1-20%) or montmorillonite (1-5%) was added to an agar media containing cadmium [44]. Likewise, in one study, it is reported that cadmium toxicity to Streptomyces bottropensis is reduced by the addition of the clay minerals vermiculite and 3% bentonite [45]. To lessen the bioavailability and mobility of metals, additional soil treatment using chemicals such as phosphate, calcium carbonate, manganese oxide, cement, and magnesium hydroxide can be applied [46].

CONCLUSION

In conclusion, despite the necessity of studying the influence of metal ions, which is usually an inhibitor to the growth rate of bacteria biodegrading toxic substances, the use of metal inhibition models to represent such an effect is uncommon and widely overlooked. The Wang model was found to be the most successful of various metal inhibition models used for the effect of mercury on the growth of an acrylamide-degrading bacterium in this investigation. The Wang model provides for the estimation of the concentration of heavy metals necessary to halt 50% of bacterial growth rate on toxics. Because the bacterium will have to deal with the toxicity of two types of toxics at once, that is the metal and the targeted toxicant to be degraded, which is also toxic to the bacterium, it is predicted that the growth rate on toxic material will be even more significantly influenced. This research has significant implications for field trial efforts pursuing acrylamide bioremediation in mercury-contaminated areas.

REFERENCES


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