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Modelling the Effect of mercury on the Growth Rate of an SDS-degrading *Pseudomonas* sp. strain Maninjau1

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ABSTRACT

The SDS-degrading bacterium *Pseudomonas* sp. strain Maninjau1 experienced significant inhibition by mercury. Observations of bacterial growth at varying mercury concentrations revealed a sigmoidal pattern, with lag periods extending from 7 to 12 hours. Increasing mercury concentrations progressively impeded growth, with a concentration of 1.0 mg/L nearly halting bacterial activity. To analyze these effects, the modified Gompertz model was employed to determine growth rates across different mercury concentrations. These rates were then subjected to several models, including the modified Han-Levenspiel, Wang, Liu, Shukor, modified Andrews, and Amor models. Among these, the Amor model failed to appropriately fit the growth curves. Statistical analysis indicated that the Shukor model performed the best, evidenced by the lowest values for Root Mean Square Error (RMSE) and Akaike's Information Criterion corrected (AICc), the highest adjusted correlation coefficient (adR^2), and values of Accuracy Factor (AF) and Bias Factor (BF) closest to unity. The parameters obtained from the Shukor model, which are μ_{max} (h^{-1}) and S_m (mg L^{-1}) and n which represent maximum growth rate, critical heavy metal ion concentration and empirical constant values were 0.187, 1.126 and 2.406, respectively. The Shukor model allows for the prediction of the critical heavy metals concentration which can completely inhibited bacterial growth. This robust modeling approach underscores the Shukor model's suitability for predicting the impact of mercury on the growth dynamics of *Pseudomonas* sp. strain Maninjau1 under toxic stress conditions.

INTRODUCTION

Detergents are recognized for their harmful effects on marine organisms. [1–3]. Previous studies have shown that anionic surfactants are toxic to many aquatic species at concentrations ranging from 0.0025 to 300 mg/L [4]. It impacted the life cycle of aquatic species and altered their behavior [5]. One study found that the oyster's digestive gland is responsive to Sodium dodecyl sulfate (SDS) exposure, resulting in adverse effects on the nutritional and metabolic processes of the oyster, ultimately reducing its survival rate [6]. Increasing the presence of anionic surfactants in water bodies will result in elevated pollution levels, leading to heightened harmful effects on invertebrates and crustaceans.

Anionic surfactant like SDS is a significant ingredient in laundry detergents and have been found in different amounts in wastewater. These chemicals in water bodies can greatly impact water quality because of their strong foaming potential and endurance. Surfactants can alter the surface tension of water, impacting the flow of oxygen to aquatic ecosystems and causing hypoxic conditions. The harmful impacts of surfactants on aquatic organisms, especially invertebrates and crustaceans, have been extensively recorded. Surfactants have the potential to harm cell membranes, causing them to become more permeable and allowing cellular contents to flow out [7–9]. Studies have shown that SDS is very harmful to *Daphnia magna,* a common freshwater invertebrate, with notable mortality occurring at doses

as low as 5 mg/L [10]. The levels of anionic surfactants in wastewater differ greatly between home and industrial origins. Domestic wastewater usually has detergent levels between 3 to 21 mg/L, while industrial sources, particularly in textile and laundry services, can have levels as high as 10,000 mg/L, which can create significant difficulties for treatment procedures. Wastewater treatment involving high concentrations of surfactants such as SDS is challenging because of their durable chemical composition and capacity to disrupt treatment procedures [11].

SDS-degrading bacteria serve as effective tools for SDS bioremediation, particularly in dilute and complex matrices such as river and seawater. However, the degradation process can be hampered by the presence of heavy metals like mercury, silver, and copper, posing significant challenges to bioremediation efforts. Understanding the threshold concentration of these metals that can inhibit bacterial growth is crucial for optimizing bioremediation strategies and ensuring effective degradation of SDS in contaminated environments. This knowledge helps in setting appropriate limits and conditions under which bioremediation can proceed effectively, despite the potential toxic effects of heavy metals.

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 [12], Wang [13], Liu [14], modified Andrews[15], Amor [16] and the Shukor model [17,18] have been utilised [19] 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}, μ, μ_{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 [20,21]. 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.

MATERIALS AND METHODS

Growth and maintenance of SDS-degrading bacterium

The SDS-degrading bacterium—*Pseudomonas* sp. strain Maninjau1 has been previously reported [20,21]. The growth of the bacterium on SDS was characterized in a microtiter plate format [22,23]. The bacterium was grown on a basal salts (BS) medium containing the followings: Na_2HPO_4, (1.39 g l^{-1}), KH_2PO_4, (1.36 g l^{-1}), KNO_3, (0.5 g l^{-1}), $CaCl_2$ (0.01 g l^{-1}), $MgSO_4$ (0.01 g l^{-1}), and $(NH_4)_2SO_4$ (7.7 g l^{-1}) and 1 mL of trace elements [20]. SDS was added into the medium (filter-sterilized) at 1.0 g l^{-1}. 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 specific growth rate on SDS was predicted using the modified Gompertz model, a common method for modeling microorganism growth on xenobiotics [24–26]. The equation (Eqn. 1) is as follows;

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

The result from the initial modeling exercise was subsequently utilized to model the impact of mercury to the growth rate.

Effect of metal on growth rate of on SDS

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

Table 1. Various growth inhibitory models.

Models	Equation	Authors
Modified Han-Levenspiel	$r = u_{max}\left(1 - \frac{C}{C_{crit}}\right)^m$	[12]
Wang	$r = \frac{u_{max}}{1 + \left(\frac{C}{K_C}\right)^m}$	[13]
Liu	$r = \frac{u_{max}K_C}{K_C + C}$	[14]
Modified Andrews	$r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[15]
Amor	$r = \frac{u_{max}C}{C + \left(\frac{C^2}{K_i}\right)}$	[16]
Shukor	$r = v_{max}\left(1 - \left(\frac{C}{S_m}\right)^n\right)$	[18]

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^2) was utilized as before [27].

RESULTS AND DISCUSSION

Growth of the bacterium at various concentrations of mercury shows a sigmoidal pattern with lag periods ranging from 7 to 12 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.

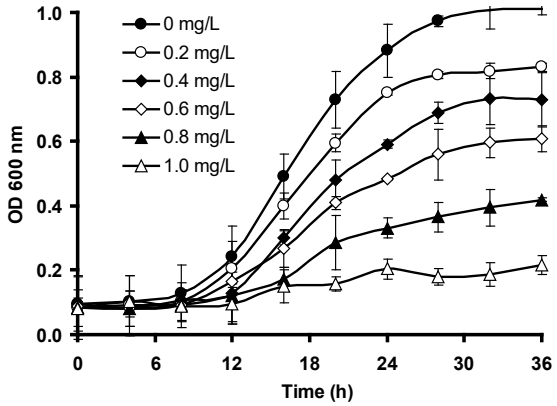

Fig. 1. Growth of *Pseudomonas* sp. strain Maninjau1 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L). The error bars represent mean ± standard deviation of triplicates.

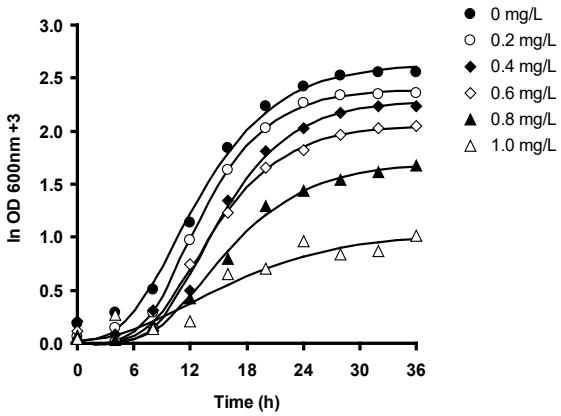

Fig. 2. Growth (log transformed) of *Pseudomonas* sp. strain Maninjau1 at 1.0 g/L SDS under various concentrations of mercury (from 0.2 to 1.0 mg/L) as modelled using the modified Gompertz model.

The growth rates at different mercury concentrations were analysed using existing metal inhibition models. Out of all the models, only the Amor model did not conform to the curve (**Figs. 3** to **7**). The revised Andrew model exhibits inadequate fitting. The statistical analysis results indicated that the Shukor model outperformed all other models 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. Error function analysis for the models fitting the inhibition of mercury to the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Wang	3	0.01	0.98	0.95	1.03	0.99	-35.67
Modified Hans-Levenspiel	3	0.01	0.99	0.98	1.02	1.00	-42.94
Liu	2	0.03	0.48	0.22	1.08	0.96	-33.53
Modified Andrews	3	0.02	0.86	0.71	2.20	0.46	-24.37
Shukor	3	0.00	1.00	0.99	1.01	1.00	-48.82

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

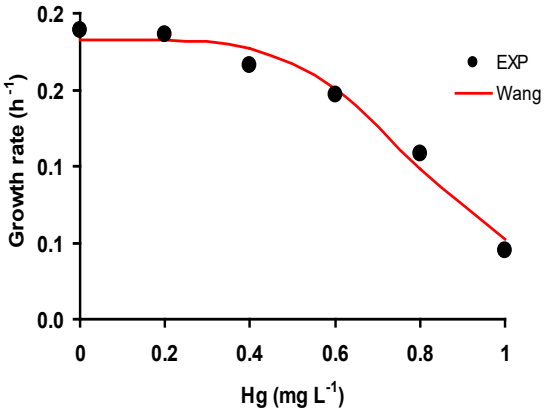

Fig. 3. The effect of mercury on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the Wang model.

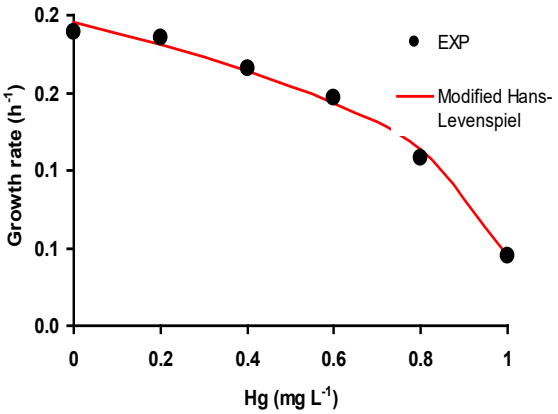

Fig. 4. The effect of mercury on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the modified Han-Levenspiel model.

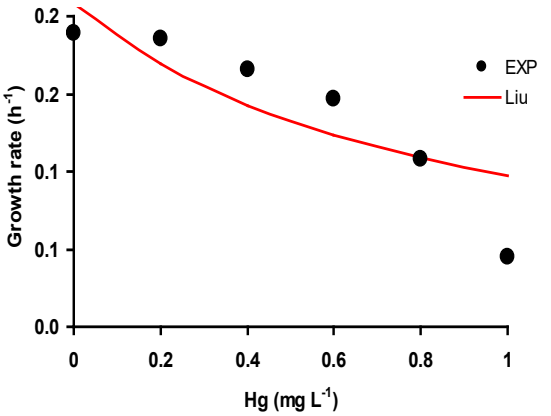

Fig. 5. The effect of mercury on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the Liu model.

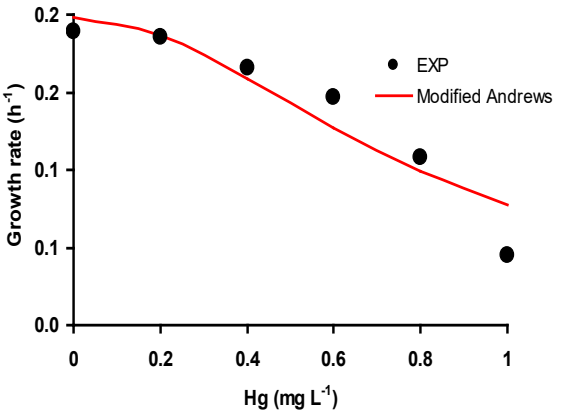

Fig. 6. The effect of mercury on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the modified Andrews model.

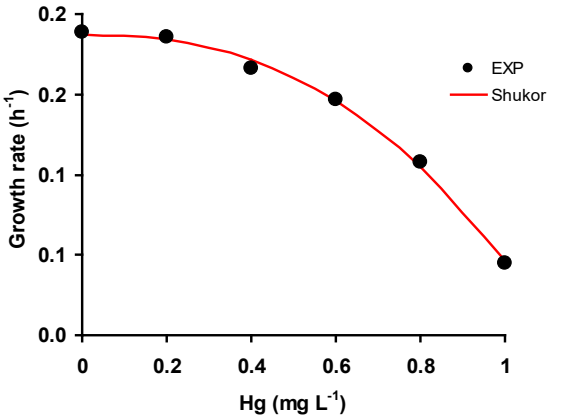

Fig. 7. The effect of mercury on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the Shukor model.

Table 3. Models' parameters for the effect of mercury on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS.

Model, parameters (95% confidence interval)	
Shukor	
μ_{max} (h^{-1})	0.187 (0.178 to 0.196)
S_m (mg L^{-1})	1.126 (1.068 to 1.183)
n	2.406 (1.824 to 2.988)
Modified Han-Levenspiel	
μ_{max} (h^{-1})	0.195 (0.180 to 0.210)
C_{crit} (mg L^{-1})	1.015 (0.983 to 1.047)
m	0.350 (0.215 to 0.485)
Wang	
μ_{max} (h^{-1})	0.182 (0.161 to 0.204)
Kc	0.828 (0.724 to 0.932
m	4.779 (1.623 to 7.934)
Liu	
μ_{max} (h^{-1})	0.208 (0.125 to 0.291)
K	0.879 (-0.332 to 2.090)

The parameters obtained from the Shukor model, which are μ_{max} (h^{-1}) and S_m (mg L^{-1}) and n which represent maximum growth rate, critical heavy metal ion concentration and empirical constant values were 0.187, 1.126 and 2.406, respectively. The Shukor model allows for the prediction of the critical heavy metals concentration which can completely inhibited bacterial growth. The Shukor model is also the best model for modelling the inhibition of mercury to the SDS-degrading bacterium *Pseudomonas* sp. strain DRY15 [18] and tributyl tin to the growth rate of *Bacillus subtilis* [17].

Current literature offers several perspectives on how heavy metals hinder the biodegradation of organic contaminants by bacteria, presenting distinct models and techniques to overcome these obstacles. Heavy metals in polluted environments can hinder the breakdown of organic contaminants such monoaromatic hydrocarbons by damaging microbial populations. Research has demonstrated that heavy metals such as zinc and nickel can significantly impede the growth and metabolic functions of bacteria like *Bacillus* sp. and *Pseudomonas* sp., which play a vital role in breaking down these pollutants. The impact can be measured quantitatively using models such as the Andrews model, which offers insights into the levels of inhibitory concentration and their influence on microbial growth rates [16].

Ongoing research is investigating the intricacies of metal inhibition in microbial biodegradation processes. The bioavailability of heavy metals in soil influences microbial degradation of organics. Methods to improve microbial resistance and biodegradation efficiency is through adapting genetically and cellularly to metal stressors [28]. The importance of creating metal-resistant bacterial strains and bioremediation techniques to address organic and metal pollutants is highlighted in these studies. Ongoing research in this area is crucial for enhancing the efficiency of bioremediation methods in environments contaminated with metals. 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. 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 [19].

Some of the key tactics include biostimulation, which is the injection of nutrients and other changes into contaminated environments to accelerate the natural biodegradation process. Microbial growth and activity can be stimulated by adjusting nutrient concentrations, which counteracts the effects of metal inhibitors [29]. Introducing metal-resistant microbial strains or consortia that are specifically adapted to break down hydrocarbons in the presence of heavy metals can significantly improve bioremediation efficiency. Metal efflux systems and enzymatic pathways are major techniques utilized by these specialised bacteria to minimize metal toxicity. Furthermore, the use of chelators or sequestrants can reduce metals' negative effects on microbial populations by binding to them and lowering their bioavailability.

Chelators like EDTA and citric acid can form stable complexes with metals, preventing them from reacting with hydrocarbon-degrading microbial enzymes [30]. To reiterate, developing genetically edited microbes with increased metal resistance and hydrocarbon breakdown capacity is a viable technique. These genetically modified bacteria can express genes that provide metal resistance or enhance metabolic pathways for hydrocarbon breakdown. Plants can also be utilized to remediate areas tainted with hydrocarbons and metals. Certain plants can accumulate heavy metals in their tissues while harboring hydrocarbon-degrading rhizosphere bacteria [31]. Solidification, stabilization, and vitrification are some of the immobilization procedures that can be used to minimize metal mobility and bioavailability in the environment. This limits their interactions with microbial groups involved in hydrocarbon degradation [32].

CONCLUSION

In conclusion, the application of metal inhibition models to analyze the impact of metal ions on the growth rates of bacteria in the presence of toxic substances remains underexplored, despite the critical importance of such research. This study examined the effect of mercury on the growth of an SDS-degrading bacterium using various metal inhibition models. Among these, the Shukor model was identified as the most effective, accurately predicting the critical concentration of heavy metals that completely inhibits bacterial growth. It is anticipated that in environments contaminated with heavy metals, the growth rate on toxic substances will be further compromised as the bacteria concurrently contend with the toxicity of both pollutants. The findings from this study are particularly valuable for field trials aiming at SDS bioremediation in locations also contaminated with mercury.

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