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Mathematical Modelling on the Effect of Mercury on the Growth Rate of *Serratia marcescens* strain DRY6 on Sodium Dodecyl Sulphate

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ABSTRACT

Sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS) is a common anionic surfactant found in various cleaning and personal hygiene products. It has both a polar "headgroup" and a hydrocarbon tail, giving it amphiphilic properties that make it effective as a detergent. However, this also makes it a major pollutant in aquatic environments. Researchers have studied the biodegradation of SDS by microorganisms, particularly bacteria, as a potential cleanup method. It has been found that mercury can significantly inhibit the degradation of SDS by the Serratia marcescens strain DRY6 bacteria. At different mercury concentrations, the bacteria exhibited sigmoidal growth with lag times of 7 to 10 hours, but overall growth was decreased with higher mercury concentrations, with a concentration of 1.0 g/L virtually stopping all growth. A modified Gompertz model was used to calculate growth rates at various mercury concentrations, and these rates were then modeled using five different models: modified Han-Levenspiel, Wang, Liu, modified Andrews, and Amor. Only three of the models (Wang, modified Han-Levenspiel, and Liu) were able to accurately fit the curve, with the Wang model performing the best statistically. The Wang model yielded estimates of 0.216 (95% confidence interval: 0.193 to 0.239) for the critical heavy metal ion concentration, 1.05 (95% confidence interval: 0.938 to 1.167) for the maximum growth rate, and 0.389 (95% confidence interval: 0.148 to 0.636) for the empirical constant , represented by C_{crit}, μ_{max} and m, respectively.

INTRODUCTION

In general, detergents are considered safe, though some of their ingredients may be harmful if ingested or used in excess. For example, bleach can irritate the skin, eyes, and lungs if inhaled, while phosphates can damage streams and rivers if not disposed of properly. Additionally, some detergents may contain fragrances, dyes, or other potentially harmful substances. One such ingredient, sodium dodecyl sulfate (also known as SDS), is a common anionic surfactant found in commercial products and cleaning detergents. There is a significant amount of evidence indicating that SDS is harmful and environmentally polluting. Studies have shown that biodegradation, a process involving bacteria, can help reduce the amount of SDS released into the environment [1,2]. Anionic surfactants are the most widely used type due to their ability to effectively clean at low temperatures in neutral solutions. They produce negatively charged ions in aqueous solution from sulfate or sulphonate groups and have numerous commercial and industrial applications [3–13]. They are also an important group of organic substances found in the marine environment due to their high solubility in both organic and inorganic chemicals [14]. Naturally produced surfactants, which are exudates from phytoplankton found beneath the surface of the water, are a primary source of anionic surfactants. These surfactants are made up of hydrophilic and hydrophobic components that can effectively interact with both polar and nonpolar structures in compound mixtures. They are used in a wide range of applications and can bring benefits to various

technological processes and biological systems by reducing the energy needed for contact and solvation in multiple heterogeneous phases [15]. Microorganisms that can degrade SDS and use it as a carbon source for growth and energy are at the forefront of bioremediation efforts for this hazardous compound in the environment [16–22].

Contaminated effluent containing hazardous metal ions can inhibit the growth and ability of bacteria to utilize toxic substances like SDS. The detrimental effects of high-saline environments and heavy metal ions on the microorganisms that break down pollutants have received significant attention in recent years because they either inhibit growth or decrease the activity of enzymes produced by the microorganisms. There are physically based methods, such as osmosis, ion exchange, and dialysis, that can remove salts, but they are too costly for use in an industrial setting. Some metals, such as those found in nuclear waste, cannot even tolerate slightly increased concentrations in the environment, despite the ability of some species to withstand and even reduce heavy metal levels. Chelating, or binding to, the metal ions causing the inhibition can help mitigate their impact and make them more manageable. The three most effective strategies for reducing the toxicity of heavy metals are precipitation, sorption, and chelation by organic and inorganic ligands. These strategies can be achieved through precipitation, sorption, and chelation of heavy metals.

Heavy metal ions, which cannot be degraded, can slow biodegradation and extend the duration of bioremediation. When accumulated by microbes to a toxic level, these ions inhibit the growth rate of bacteria, as they cannot be destroyed. Their inhibitory effects can be studied by adjusting the model of substrate inhibition for harmful ion presence. Numerous models, including a modified version of the Han-Levenspiel [23], Andrews [27] Wang [25], modified Amor [26], Liu [24], and the Shukor model [28] have been utilised to assess the outcome of heavy metal on the bacterial degradation of toxic materials. From these, models' inhibition related constants can be found.
Since fish and fish products may absorb high concentrations of mercury without showing any obvious symptoms of poisoning, they are frequently consumed by those who are mercury intoxicated. It is believed that fish may accumulate up to one million times the amount of mercury found in polluted water. Mercury poisoning from fish was especially damaging in the 1950s in the Japanese cities of Minamata and Niigata. Fish caught in these waters was found to be very toxic due to the discharge of methylmercury from an industrial site into the rivers and ocean. Approximately 600 people in Minamata died as a direct result of the outbreak, with over 3,000 cases documented nationwide.

Consuming mercury-treated food is another potential route of exposure. In the so-called Basra poison grain catastrophe of 1972, for instance, almost 400 individuals died from mercury poisoning after eating plant-growing grain that had been sprayed with methylmercury. There were almost 6,000 confirmed cases during this outbreak. Mercury is used in many items, including explosives, fluorescent lights, laboratories, dental amalgams, and batteries, but it may also be poisonous if ingested, inhaled, or (in the case of dimethylmercury) absorbed through the skin. There is a higher danger of inhalation poisonings in activities like coal burning and gold mining.

People should be evacuated from areas where mercury is being handled because of the risk of poisoning from inhaling mercury compounds. Mercury's effects inside the body are complex and multifaceted, with many of the underlying mechanisms still a mystery. However, the central nervous system, the kidneys, and the endocrine glands are particularly vulnerable to mercury's effects. Varied types of mercury can have different effects, but all of them have the potential to have devastating teratogenic consequences on developing embryos. Exposure to mercury, for instance, has been linked to impaired brain development in children. This is because mercury blocks the production of myelin sheaths, which protect nerve cells. In addition, catechol-o-methyl transferase relies on S-adenosyl-methionine for its breakdown, and mercury is considered to inhibit this enzyme's function. Excessive levels of catecholamines in the body might lead to the development of the associated symptoms.

There are few studies available that examine the effect of heavy metals on microbial growth, as most studies on this topic use primary models rather than secondary models. In this work, an SDS-degrading bacterium was isolated and it was found that heavy metals including mercury, silver, and mercury significantly inhibited its growth [29,30]. Using various inhibition models, the aim of this study was to investigate the effect of mercury on the growth rate of this bacterium when grown on SDS. By employing a number of different inhibition models, the purpose of this work is to investigate the impact that mercury has on the pace at which this bacterium grows when it is grown on SDS.

MATERIALS AND METHODS

Growth of SDS-degrading bacterium

Serratia marcescens strain DRY6 was previously isolated and characterized [31]. Studies on the effect of heavy metal to the growth of the bacterium on SDS utilized the microtiter plate format [32,33]. The growth medium was as follows: Na_2HPO_4, (1.39 g l^{-1}), KNO_3, (0.5 g l^{-1}), KH_2PO_4, (1.36 g l^{-1}),$MgSO_4$ (0.01 g l^{-1}), $CaCl_2$ (0.01 g l^{-1}), and $(NH_4)_2SO_4$ (7.7 g l^{-1}) [29]. Then, SDS was filter sterilized using a filter syringe (0.2 µm) and added into the cooled medium to the final concentration of 1000 mg/L. The Corning® microplates were incubated under vacuum at 30 degrees Celsius, and the absorbance at 600 nm was measured (BioRad reader, model 680, Richmond, CA).

Growth model on SDS

The modified Gompertz model, which is often used for modeling the growth of microorganisms on xenobiotics, was chosen to predict the maximum specific growth rate that could be achieved on SDS [34–36]. The equation is as follows;

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

Following the completion of this basic modeling activity, the value that was acquired was then used to predict the influence of metal as follows;

Effect of metal on growth rate of on SDS

The models utilized in this study is as follows;

Models	Equation	Authors
Modified Han-Levenspiel	$r = u_{max}\left(1 - \frac{C}{C_{crit}}\right)^m$	[23]
Wang	$r = \frac{u_{max}}{1 + \left(\frac{C}{K_C}\right)^m}$	[25]
Liu	$r = \frac{u_{max}K_C}{K_C + C}$	[24]
Modified Andrews	$r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[27]
Shukor	$r = \upsilon_{max}\left(1 - \left(\frac{C}{S_m}\right)^n\right)$	[28]
Amor	$r = \frac{u_{max}C}{C + \left(\frac{C^2}{K_i}\right)}$	[26]

Fitting of the data

The Marquardt approach was employed to fit the nonlinear equations, and the CurveExpert Professional program (Version 1.6) was utilized. The algorithm aims to find the method that results in the minimum sum of squares between the predicted and measured values. The software automatically calculates the initial values using a method that considers the steepest ascent.

Statistical analysis

In order to determine the optimal model, a variety of statistical methods were utilized, including the corrected Akaike Information Criterion (AICc), Root-mean-square Error (RMSE), bias factor (BF), accuracy factor (AF), and modified or adjusted coefficient of determination (adjR^2). These methods were applied in the same manner as before [37].

RESULTS AND DISCUSSION

Sodium dodecyl sulfate (SDS), an anionic surfactant, has numerous applications and is often released into the environment through water and soil. However, due to its toxic effects on various species, it is important to eliminate SDS from the environment. One strategy for reducing SDS toxicity is bioremediation, which is the use of living organisms to clean up and remove contaminants from the environment. Currently, bacteria are the preferred method for bioremediating SDS contamination in soil and water sources due to their efficiency and low cost. As the need for SDS disposal increases, it is important to utilize more effective strains of bacteria to ensure environmental protection [39]. According to research conducted by Rebello et al., the breakdown of SDS is often carried out by *Pseudomonas* species in both soil and aquatic environments.

The *Pseudomonas* genus, which is widespread and highly effective at degrading hazardous chemicals, is particularly useful for reducing the impact of industrial effluents, including SDS [40]. The SDS-degrading capacity of the *Serratia* genus is not often reported but this genus is often found in human-impacted environment. In a study examining the growth of Pseudomonas in the presence of different doses of mercury, a sigmoidal pattern was observed, with lag periods ranging from seven to ten hours (**Fig. 1**).

The total growth of the bacterium was hindered as the concentration of mercury increased, with a concentration of 1.0 g/L nearly completely inhibiting growth. To obtain growth rates at various concentrations of mercury, the modified Gompertz model was applied (**Fig. 2**). This model showed a close fit to the data and was consistent with its predictions. The model also demonstrated that an increase in the concentration of mercury resulted in a decrease in growth rates and an increase in the lag time, even if the lag period was extended.

Fig. 1. The growth of *Serratia marcescens* strain DRY6 on SDS at a concentration of 1.0 g/L in the presence of mercury at various concentrations ranging from 0.2 to 1.0 mg/L was studied. The mean and standard deviation of the triple measurements are represented by the error bars in the graph.

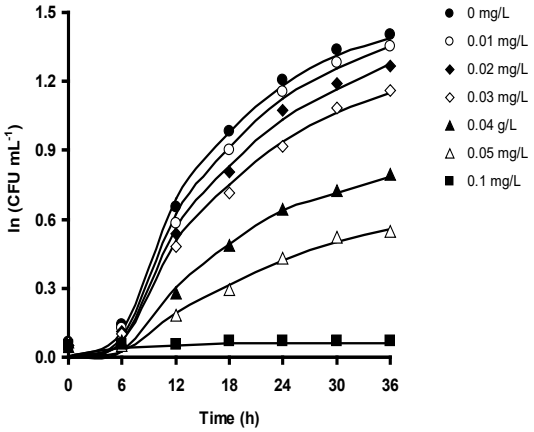


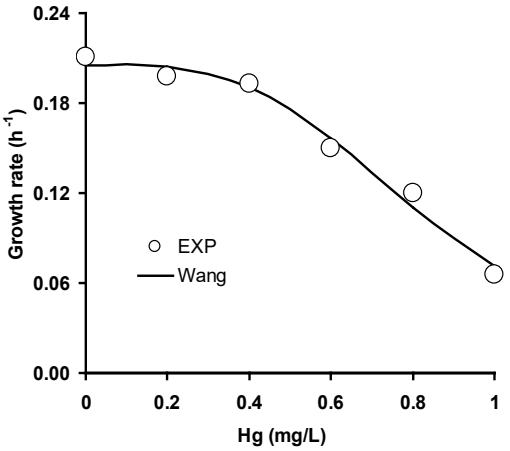
Fig. 1. The growth of *Serratia marcescens* strain DRY6 on SDS at a concentration of 1.0 g/L at various concentrations of mercury (ranging from 0.2 to 1.0 mg/L) was modeled using the modified Gompertz model. The growth is depicted as a log transformation in the graph.

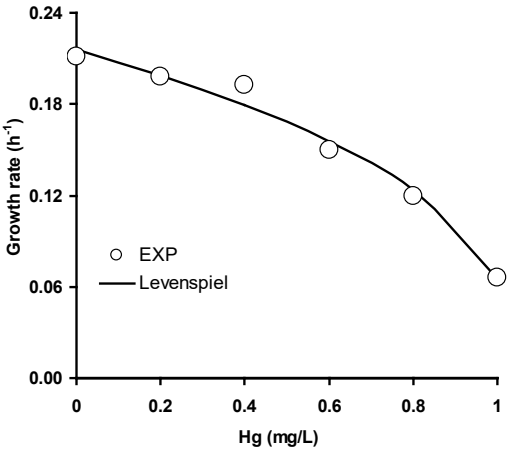
Afterwards, the growth rates at various concentrations of mercury were modeled using a variety of metal inhibition models. With the exception of the Amor model, all of the other models were able to fit the curve. However, the Amor model did not fit the curve well (**Figs. 3** to **5**). In contrast, the Wang and modified Han-Levenspiel models showed acceptable fitting, while the Liu model displayed poor fitting. A statistical analysis showed that the Wang model was the most accurate representation of the data, as it produced the fewest outliers. In terms of RMSE and AICc, the Wang model also had the highest adjusted correlation coefficient (adR^2) and values of AF and BF closest to unity (**Table 1**).

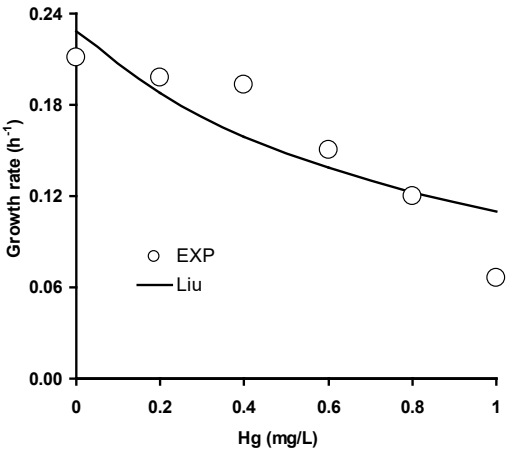
Table 1. Error function analysis for all models.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Wang	3	0.01	0.99	0.98	1.03	0.99	-36.75
Levenspiel	3	0.01	0.99	0.98	1.03	0.99	-37.87
Liu	2	0.04	0.40	0.09	1.10	0.94	-28.93
Andrews	3	0.05	0.40	-0.21	2.32	0.43	-12.93
Amor	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Shukor	3	0.00	1.00	0.99	1.02	0.99	-44.13

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







The Shukor model, which can be accessed here, enables the prediction of the critical heavy metal ion concentration that can fully suppress bacterial growth, which is highly valuable for translating laboratory data to the field. The results of using this model yielded the following values for the parameters C_{crit}, μ_{max} and m: 0.209 mg/L, 0.103 per h, and 1.530, respectively. These values reflect the critical concentration of heavy metals (in mg/L), the maximum growth rate (per h), and the empirical constant values, respectively. In addition to these capabilities, the Shukor model can also provide insight into the relationship between heavy metal concentrations and bacterial growth.

Table 2. Parameter values for growth rate inhibition models.

Model	Value	95% Confidence Interval
Shukor		
μ_{max}	0.209	0.193 to 0.225
C_{crit},	0.103	0.096 to 0.110
n	1.530	1.104 to 1.957
Wang		
μ_{max}	0.199	0.185 to 0.212
K_C	0.057	0.050 to 0.064
m	4.642	2.442 to 6.841
Modified Han–Levenspiel		
μ_{max}	0.214	0.195 to 0.233
C_{crit},	0.101	0.098 to 0.103
m	0.587	0.312 to 0.861
Liu		
μ_{max}	0.233	0.145 to 0.321
K_C	0.059	-0.020 to 0.137
Amor	n.a.	
μ_{max}	n.a.	
K_i	n.a.	
Modified Andrews		
μ_{max}	0.014	-0.007 to 0.034
K_s	0.059	-0.049 to 0.166
K_i	-11432494.153	-1210244909640420 to 1210244886775432

There have been few studies that have looked into the effect of heavy metals on the population growth of bacteria living on toxic compounds. For instance, research has shown that zinc and nickel significantly reduce the rate of biodegradation of monoaromatic hydrocarbons by *Bacillus* sp. and *Pseudomonas* sp., and the Andrews model has been effectively used to study the impact of these heavy metals on the degradation rate. Despite the importance of this research, given the presence of heavy metals in polluted streams, the use of metal inhibition models is underrepresented in the scientific literature.

One possible explanation for the inhibitory effects of heavy metals on bacterial growth is that they decrease enzyme activity by binding to critical enzyme functional groups, such as the sulfhydryl group, which is often located in the active regions of enzymes. This binding may interfere with the enzyme's ability to perform its function and lead to the inhibition of biodegradation. Overall, understanding the mechanisms by which heavy metals affect bacterial growth is essential for predicting and mitigating their environmental impact [41].

There are several approaches that can be taken to address the issue of heavy metals inhibiting biodegradation. One solution is to use treatment additives, such as calcium carbonate, manganese oxide, cement, phosphate, and magnesium hydroxide, which can limit the bioavailability and mobility of metals and make it easier to clean up metal pollution. Another approach is to utilize the minerals found in clay, as clay minerals have been shown to be effective at reducing the bioavailability and toxicity of metals in the environment. For example, the toxicity of cadmium was reduced when kaolinite (1-20%) or montmorillonite (1-5%) was added to agar media containing cadmium for use by yeasts, bacteria, and an actinomycete. This cadmium-containing agar medium was used to culture yeasts, bacteria, and an actinomycete.

Overall, finding ways to mitigate the negative effects of heavy metals on biodegradation is crucial for managing and cleaning up metal pollution [43]. Similarly, Kamel found that 3% bentonite and vermiculite in solution testing were effective at reducing the toxicity of 150 mg total cadmium/L to *Streptomyces bottropensis*. However, while kaolinite was also successful in reducing the toxicity of cadmium, it required a higher

concentration (6% rather than 3%) and overall offered less protection compared to the other clays. These findings suggest that different types of clay minerals may have varying degrees of effectiveness in mitigating the toxic effects of heavy metals, and it may be worthwhile to explore their potential for use in cleaning up metal pollution [44]. One approach that may be used to address the problem of heavy metals inhibiting biodegradation is the inoculation of metal-resistant bacteria. This involves introducing bacteria that are resistant to heavy metals into the environment, where they can reduce the amount of bioavailable metal. By decreasing the amount of metal present, the process of biodegradation is accelerated, which can help to mitigate the negative effects of hazardous metals. This approach can be effective at reducing the amount of bioavailable metal in the environment and speeding up the process of biodegradation when a hazardous metal is present. Additionally, inoculation of metal-resistant bacteria can be a useful strategy for addressing the issue of heavy metals inhibiting biodegradation.

By reducing the amount of bioavailable metal in the environment, this approach can help to accelerate the process of biodegradation and mitigate the negative effects of hazardous metals [45]. One way to improve the effectiveness of acrylamide breakdown is to combine a primary bacterial degrader with a bacterium that is resistant to metals. This approach was demonstrated in a soil microcosm experiment, in which a cadmium-resistant Pseudomonas H1, which accumulates cadmium in the cell, and 2,4-D-degrading bacteria were introduced to soil contaminated with both cadmium (60 mg total cadmium/kg) and 2,4-D (500 mg/kg). This resulted in an enhanced degradation efficiency of the xenobiotic. This example illustrates the potential benefits of using a combination of metal-resistant bacteria and primary degraders to improve the effectiveness of biodegradation in contaminated environments [46].

CONCLUSION

To summarize, metal inhibition models have not been widely used to predict the effect of metal ions on the growth rate of bacteria exposed to hazardous substances, despite the importance of this research. However, in this study, several metal inhibition models were used to investigate how mercury would impact the growth of bacteria capable of digesting SDS. It was found that the Wang model was the most accurate in describing this phenomenon. This model allows us to estimate the critical concentration of heavy metals required to completely inhibit bacterial growth. It is likely that the presence of heavy metals will significantly impact the rate of biodegradation of hazardous substances. This is because bacteria must be able to survive the toxicity of both the heavy metals and the toxicants they are attempting to break down. Future field trial initiatives aiming to incorporate SDS bioremediation into mercury-polluted areas may find the results of this study valuable in their efforts.

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