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Silver Impact on the Growth Rate of an SDS-degrading *Pseudomonas* sp. strain Maninjau1 Explored using Predictive Models

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ABSTRACT

The use of silver nanoparticles in fabrics in conjunction with detergent in washing may release silver ions that can inhibit detergent remediation by microorganisms. The SDS-degrading bacterium, *Pseudomonas* sp. strain Maninjau1, showed prominent inhibition in the presence of silver ion. When exposed to varying silver concentrations, the bacterium's growth followed a sigmoidal pattern, exhibiting lag periods ranging from 2.5 to 5.5 hours, as modeled using the modified Gompertz model. Increasing the silver concentrations progressively slowed bacterial growth, with concentrations as low as 1.0 mg/L halting bacterial growth rate altogether. To assess these effects, the modified Gompertz model was used to determine growth rates across different silver concentrations. The results were then evaluated against several models, including the modified Han-Levenspiel, Wang, Liu, Shukor, modified Andrews, and Amor models. The Amor model did not adequately fit the growth curves. Statistical analysis revealed that the modified Han-Levenspiel model performed best, as indicated by the lowest RMSE and AICc values, the highest adjusted correlation coefficient (adR^2), and AF and BF values closest to unity. The Liu, Shukor, modified Andrews, and Wang models followed in descending order of performance. The parameters obtained from the modified Han-Levenspiel model, which are μ_{max} (h^{-1}) and C_{crit} (mg L^{-1}) and m which represent maximum growth rate, critical heavy metal ion concentration and empirical constant values were 0.196, 1.1134 and 0.632, respectively. The modified Han-Levenspiel model effectively predicts the critical concentrations of heavy metals that can completely inhibit bacterial growth. This robust modeling approach highlights the model's suitability for forecasting the impact of silver on the growth dynamics of *Pseudomonas* sp. strain Maninjau1, particularly under conditions of toxic stress.

INTRODUCTION

An anionic surfactant such as SDS is a key component in laundry detergents and has been detected in varying concentrations in wastewater. The presence of these compounds in water bodies can significantly affect water quality due to their high foaming capacity and persistence. Surfactants can modify the surface tension of water, affecting the oxygen flow to aquatic habitats and leading to hypoxic conditions. Surfactants have been thoroughly documented to have negative effects on aquatic creatures, particularly invertebrates and crustaceans. Surfactants can damage cell membranes, making them more permeable and leading to the leakage of cellular contents [1–3]. Detergents are known for their detrimental impact on marine species [4–6]. Previous studies have shown that anionic surfactants are toxic to many aquatic species at concentrations ranging from 0.0025 to 300 mg/L [7].It affected the life cycle of aquatic creatures and changed their behavior [8].

A study discovered that the oyster's digestive gland reacts to Sodium dodecyl sulfate (SDS) exposure, causing negative impacts on the nutritional and metabolic functions of the oyster, leading to a decrease in its survival rate [9]. Augmenting the concentration of anionic surfactants in water will cause increased pollution levels, resulting in more severe impacts on invertebrates and crustaceans.

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Research has demonstrated that SDS is very toxic to *Daphnia magna*, a prevalent freshwater invertebrate, causing significant death even at concentrations as low as 5 mg/L [10]. The amount of surfactants found in residential and industrial wastewater sources can vary significantly. In household wastewater the detergent levels typically fall between 3 to 21 mg/L. However, in wastewater, from industries like textiles and laundry services the surfactant concentrations can soar as high as 10,000 mg/L. These elevated levels present challenges for treating wastewater. Managing such high surfactant levels poses difficulties during the wastewater treatment process due to their chemical makeup and their ability to disrupt treatment methods. For instance, substances like sodium dodecyl sulfate (SDS) are particularly stubborn to break down.

SDS hinder the processes crucial for effective wastewater treatment. Adjustments to treatment approaches, such, as improving biodegradation capabilities or incorporating filtration methods are often required to tackle these elevated concentrations of anionic surfactants [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 silver, 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.

Silver, in its various forms such as silver, silver nanoparticles, and ionic silver, exhibits different levels of toxicity towards microorganisms, animals, and humans. Toxicity levels and types are primarily determined by the form of silver and the extent of exposure. Silver has been highly esteemed for its properties over the years. Its main mechanism of action against microorganisms involves interacting with thiol groups in enzymes and proteins, leading to alterations and disruptions in cellular functions. Silver exposure can disrupt the processes of bacteria and fungi, potentially leading to cell death. Silver nanoparticles are highly efficient because of their large surface area. Discover applications in coatings for medical devices and water purification systems. Concerns have been raised about the development of resistance to silver as an antimicrobial agent, which could result in the emergence of 'superbugs' that are more difficult to treat [12–17].

Silver toxicity in animals primarily manifests through oral ingestion and inhalation exposure. Research on aquatic organisms such as fish has shown that the build-up of silver in their gills can result in respiratory problems and potentially fatal consequences [18]. Silver nanoparticles are concerning because their small size allows them to easily penetrate biological barriers and accumulate in various organs. Nanoparticle exposure has been linked to lung inflammation, liver toxicity, and altered responses in mammals such as laboratory rodents. Toxicity levels are influenced by factors like nanoparticle size, coating, concentration, and duration of exposure. People primarily encounter silver through skin contact, ingestion, or inhalation. When in work environments or using medical products that contain silver. Extended contact with silver can result in argyria. Gray discoloration of the skin and tissues caused by accumulation. Although argyria is typically considered non-life-threatening, excessive absorption of silver can lead to more severe health complications such as neurological disorders, kidney damage, and liver injury. Prudent management is essential when utilizing silver in consumer goods and medical devices to avoid health implications, as silver can be absorbed through various pathways [12–17,19–22].

Toxic metal ions in contaminated wastewater have been proven to hinder bacterial growth and the processing of dangerous compounds. Heavy metals specifically impede the biodegradation process and hence limit bioremediation attempts. Heavy metal ions are non-degradable, unlike most other inhibitors. When bacteria acquire dangerous amounts of substances, it greatly hinders their growth rate. Given the persistence of heavy metals in the environment and organisms, it is essential to address their influence. To comprehend and forecast the inhibitory impacts of these metals, one useful method is to adjust the substrate inhibition model. By modifying this model, researchers can enhance their analysis and measurement of the inhibitory impacts induced by hazardous ions, resulting in more efficient approaches for treating contaminated wastewater [23–29].

Metal inhibition models include the modified Han-Levenspiel [30], Wang [31], Liu [32], modified Andrews[33], Amor [34] and the Shukor model [35,36] have been utilised [37] to evaluate the result of heavy metal on the bacterial growth rate on toxic substances. 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. Silver has been demonstrated to strongly inhibited the growth of an SDS-degrading bacterium [38,39]. This study aims to investigate how silver impacts the growth rate of this bacterium on SDS by utilizing several inhibitory models.

MATERIALS AND METHODS

Media for the Growth and maintenance of SDS-degrading bacterium

The growth of the SDS-degrading bacterium—*Pseudomonas* sp. strain Maninjau1 [38,39] on SDS was characterized in a microtiter plate format as before [40,41]. The bacterium was grown on a SDS as a sole carbon source on 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}) [38]. SDS was added into the medium (filter-sterilized) at 1.0 g l^{-1}. The microplates (Corning® microplate) were incubated for 48 h sealed at 30 °C and the growth of bacterium on SDS was read at 600 nm at suitable time intervals (BioRad reader, model 680, Richmond, CA).

Modified Gompertz model

The modified Gompertz model was used to predict the specific growth rate on SDS, a standard approach for predicting microorganism growth on xenobiotics [42–44]. The equation is as follows;

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

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The result from the initial modeling exercise was subsequently utilized to model the impact of metal in the following manner;

Metal inhibition models

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

Table 1. Various growth inhibitory models.

Models	Equation	Authors
Modified Han-Levenspiel	$r = u_{max}\left(1 - \frac{C}{C_{crit}}\right)^m$	[30]
Wang	$r = \frac{u_{max}}{1 + \left(\frac{C}{K_C}\right)^m}$	[31]
Liu	$r = \frac{u_{max} K_C}{K_C + C}$	[32]
Modified Andrews	$r = \frac{u_{max} C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[33]
Amor	$r = \frac{u_{max} C}{C + \left(\frac{C^2}{K_i}\right)}$	[34]
Shukor	$r = v_{max}\left(1 - \left(\frac{C}{S_m}\right)^n\right)$	[36]

Nonlinear regression Software

The nonlinear equations were optimized using a Marquardt algorithm in CurveExpert Professional software (Version 1.6). The algorithm seeks the optimal approach that reduces the total sum of the squares between anticipated and observed values. The software automatically determines the initial values using the steepest ascent algorithm.

Error function analysis

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 [45].

RESULTS AND DISCUSSION

Growth of the bacterium at various concentrations of silver shows a sigmoidal pattern with lag periods ranging from 2.5 to 5.5 h (**Fig. 1**). As the concentration of silver 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 silver, the modified Gompertz model was utilized (**Fig. 2**), which shows close fitting to the model.

The model also shows that as the concentration of silver was increased, this led to a decrease in growth rates and an increase in lag period as well.

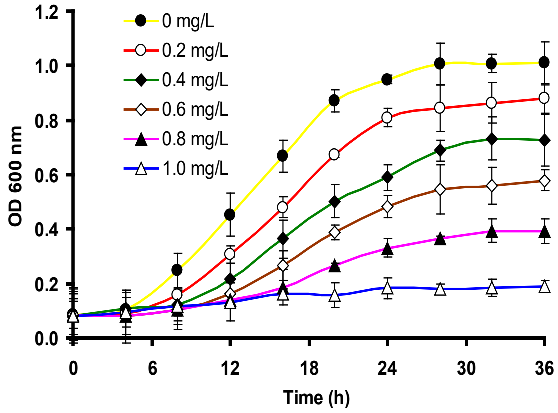

Fig. 1. Growth of *Pseudomonas* sp. strain Maninjau1 at 1.0 g/L SDS under various concentrations of silver (from 0.2 to 1.0 mg/L). The error bars represent mean ± standard deviation of triplicates.

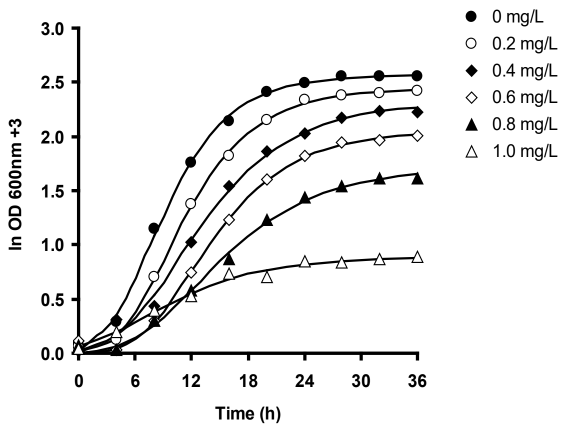

Fig. 2. Growth (log transformed) of *Pseudomonas* sp. strain Maninjau1 at 1.0 g/L SDS under various concentrations of silver (from 0.2 to 1.0 mg/L) as modelled using the modified Gompertz model.

The growth rates at different silver 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 statistical analysis results indicated that the modified Han-Levenspiel 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. This is followed in descending order by the Liu, Shukor, modified Andres and Wang model (**Table 2**).

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Table 2. Error function analysis for the models fitting the inhibition of silver to the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS.

Model	p	adR^2	AF	BF	AICc
Modified Han-Levenspiel	3	0.98	1.02	1.00	-40.19
Liu	2	0.75	1.06	0.97	-39.83
Shukor	3	0.96	1.03	0.99	-37.36
Modified Andrews	3	0.90	2.21	0.46	-30.61
Wang	3	0.89	1.05	0.98	-30.40

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

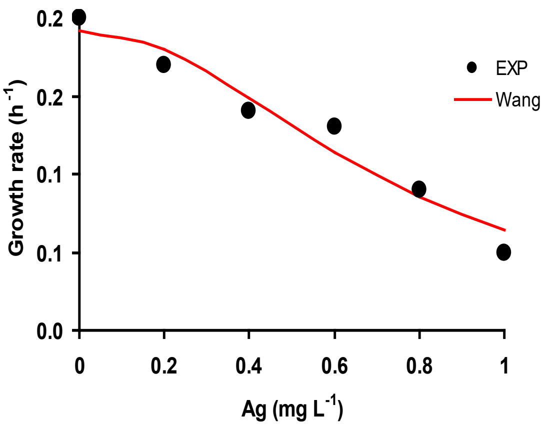

Fig. 3. The effect of silver on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the Wang model.

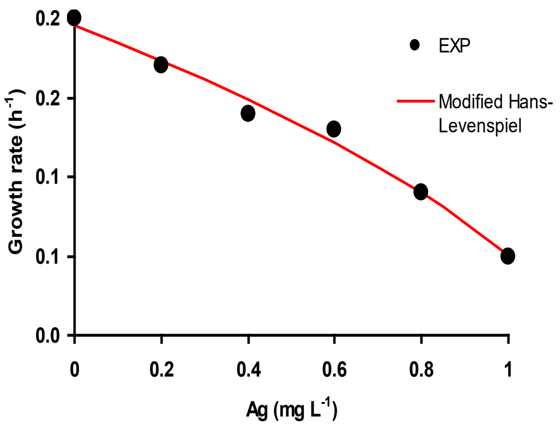

Fig. 4. The effect of silver on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the modified Han-Levenspiel model.

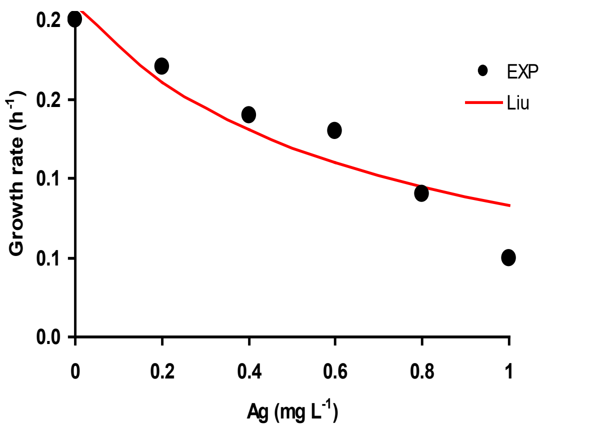

Fig. 5. The effect of silver on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the Liu model.

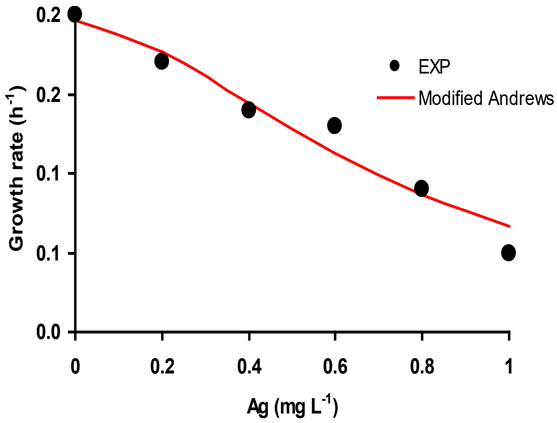

Fig. 6. The effect of silver on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the modified Andrews model.

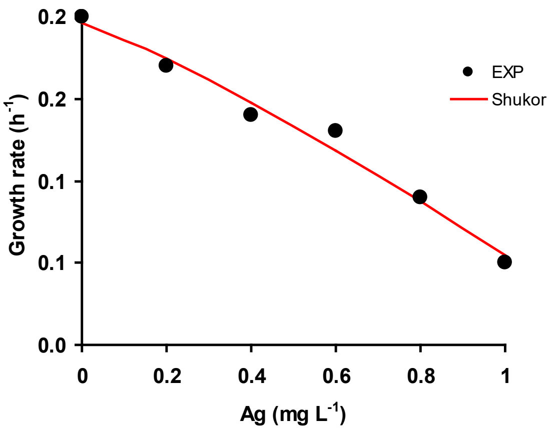

Fig. 7. The effect of silver on the growth rate of *Pseudomonas* sp. strain Maninjau1 on SDS as modelled using the Shukor model.

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

Model, parameters (95% confidence interval)	
Modified Han-Levenspiel	
μ_{max} (h^{-1})	0.196 (0.175 to 0.216)
C_{crit} (mg L^{-1})	1.134 (0.852 to 1.416)
m	0.632 (0.199 to 1.065)
Liu	
μ_{max} (h^{-1})	0.209 (0.154 to 0.263)
K	0.662 (0.123 to 1.202)
Shukor	
μ_{max} (h^{-1})	0.196 (0.168 to 0.224)
S_m (mg L^{-1})	1.324 (0.979 to 1.668)
n	1.169 (0.484 to 1.853)
Modified Andrews	
μ_{max} (h^{-1})	0.990 (-8.758 to 10.738)
K_s (mg L^{-1})	0.113 (-1.178 to 1.405)
K_i (mg L^{-1})	5.041 (-45.327 to 55.409)
Wang	
μ_{max} (h^{-1})	0.990 (-8.758 to 10.738
Kc	5.041 (-45.327 to 55.409
m	0.113 (-1.178 to 1.405

The parameters obtained from the Modified Han-Levenspiel model, which are μ_{max} (h^{-1}) and C_{crit} (mg L^{-1}) and m which represent maximum growth rate, critical heavy metal ion concentration and empirical constant values were 0.196, 1.1134 and 0.632, respectively. The modified Han-Levenspiel model allows for the prediction of the critical heavy metals concentration which can completely inhibited bacterial growth. The modified Han-Levenspiel model is also the best model for modelling the inhibition of silver to the SDS-degrading bacterium *Pseudomonas* sp. strain DRY15 [36] and tributyl tin to the growth rate of *Bacillus subtilis* [35], modelling the effect of copper on the molybdenum-reduction rate of the Antarctic bacterium *Pseudomonas* sp. strain DRY1 [46], modelling the effect of Fe^{2+} concentration on the kinetics of biohydrogen production [47], modelling the effect of copper on the growth rate of *Enterobacter* sp. strain Neni-13 on SDS [48], modelling the inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by *Pseudomonas* sp. mutant [37], modelling the effect of copper nanoparticles on the fermentative hydrogen production by *Enterobacter cloacae* and *Clostridium acetobutylicum* [49], modelling the effect of NaCl on the hydrogen production of a marine bacterium, *Vibrio tritonius* [50] and modelling the inhibitory effect of Cu(II) on *Nostoc muscorum* biomass growth and nitrate uptake [51].

Current literature provides diverse perspectives on how heavy metals inhibit the biodegradation of organic contaminants by bacteria, presenting a range of models and techniques to address these challenges. In polluted environments, heavy metals can hinder the breakdown of organic contaminants, such as monoaromatic hydrocarbons, by damaging microbial populations. Research has shown that heavy metals like zinc and nickel can significantly impede the growth and metabolic functions of bacteria, such as *Bacillus* sp. and *Pseudomonas* sp., which are crucial for pollutant degradation. The Andrews model, for instance, offers quantitative insights into inhibitory concentration levels and their effects on microbial growth rates, helping researchers understand and predict the impacts of heavy metals on these bacterial functions [34].

Current research is examining the complexities of metal inhibition in microbial biodegradation processes. Soil's heavy metal bioavailability affects the breakdown of organic matter by microorganisms. Enhancing microbial resistance and biodegradation efficiency can be achieved by genetically and cellularly responding to metal stresses [52]. These findings emphasize the significance of developing metal-resistant bacterial strains and bioremediation methods to combat organic and metal pollution. Continued research in this field is essential for improving the effectiveness of bioremediation techniques in metal-contaminated areas. The literature lacks adequate representation of metal inhibition models, despite their significance in studying the presence of heavy metals in polluted streams combined with organic pollutants. Heavy metals attach to crucial functional groups of enzymes, like the sulfhydryl group commonly located at enzyme active sites, which likely causes inhibition [37].

Key approaches in reducing the inhibitory effect of silver involve biostimulation, which is injecting nutrients and making other alterations in polluted areas to speed up the natural biodegradation process. Modifying nutrient concentrations can enhance microbial growth and activity, hence mitigating the impact of metal inhibitors including silver [53]. Silver-resistant microbial strains or consortia designed to degrade hydrocarbons in the presence of heavy metals can greatly enhance the effectiveness of bioremediation. Specialized bacteria use metal efflux systems and enzymatic mechanisms to reduce metal toxicity. Chelators or sequestrants can decrease the harmful impact of metals on microbial populations by binding to them and reducing their bioavailability. Chelators such as EDTA and citric acid can create stable complexes with metals, which inhibits their interaction with hydrocarbon-degrading microbial enzymes [54].

Developing genetically modified microorganisms with enhanced silver tolerance and the ability to break down organic pollutants is a feasible method. These genetically modified bacteria can activate genes that offer resistance to metals or improve metabolic pathways for breaking down hydrocarbons. Plants can be used to clean up regions contaminated with hydrocarbons and metals. Some plants can collect heavy metals in their tissues and host hydrocarbon-degrading bacteria in their root zone [55]. Immobilization methods such as solidification, stabilization, and vitrification can reduce silver mobility and bioavailability in the environment. This restricts their engagement with microbial communities responsible for breaking down organic pollutants [56].

CONCLUSION

In conclusion, the application of metal inhibition models to evaluate the effects of silver ions on bacterial growth rates in the presence of toxic substances remains underexplored, despite the vital importance of such research. This study analyzed the impact of silver on the growth of an SDS-degrading bacterium using various metal inhibition models. The modified Han-Levenspiel model was identified as the most effective, accurately predicting the critical concentration of heavy metals that completely inhibits bacterial growth. It is expected that in environments contaminated with heavy metals, bacterial growth rates on toxic substances will be further compromised as the bacteria simultaneously contend with the toxicity of both pollutants. The findings from this study are particularly valuable for field trials focused on SDS bioremediation in areas also contaminated with silver.

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