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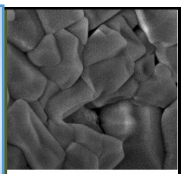

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SEM of seaweed

Inhibitory Effect of Copper on the Growth Rate of *Serratia marcescens* strain DRY6 on SDS

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ABSTRACT

The anionic surfactant known as sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS) is found in a wide variety of products designed for cleaning and personal hygiene. Because of the combination of its hydrocarbon tail and its polar "headgroup," the molecule possesses the amphiphilic qualities that make it suitable for use as a detergent. Due to this it is a major pollutant in aquatic bodies. One of the most researched types of cleanup is biodegradation by microorganisms, particularly bacteria. Copper has a profound effect in inhibiting the degradation of SDS by the bacteria *Serratia marcescens* strain DRY6. Under different copper concentrations, the SDS-degrading bacteria grew in a sigmoidal manner with lag times of 7 to 10 hours. Overall growth was decreased when the concentration of copper was raised, with 1.0 g/L virtually completely stopping bacterial growth. Rates of expansion at various copper concentrations were calculated using a modified Gompertz model. Following the modification of the Gompertz model, the growth rates were modeled using the modified Han-Levenspiel, Wang, Liu, modified Andrews, and the Amor models. Only three of the five models (Wang, modified Han-Levenspiel, and the Liu models) were able to match the curve; the modified Andrews and Amor models did not. As for model fit, the Wang and modified Han-Levenspiel models perform admirably, but the Liu model performs poorly. The Wang model performed best statistically, with the lowest RMSE and AICc values, the greatest adjusted correlation coefficient (adR2), and AF and BF values closest to unity. The Wang model yielded estimates of 0.216 (95% confidence interval: 0.193 to 0.239), 1.05 (95% confidence interval: 0.938 to 1.167), and 0.389 (95% confidence interval: 0.148 to 0.636) for the critical heavy metal ion concentration (g/l), maximum growth rate (g/l h), and empirical constant.

INTRODUCTION

In average, detergents are regarded as harmless, although certain of their constituents can be harmful if consumed or used in high quantities. Many detergents include bleach, which, if breathed, can irritate the skin, eyes, and lungs. Other detergents may include phosphates, which, if not handled of correctly, can damage streams and rivers. Moreover, certain detergents may include scents, colors, and other potentially harmful substances. An anionic surfactant that is frequently found in commercial products and cleaning detergents, sodium dodecyl sulfate, sometimes known as SDS, can be abbreviated as SDS. There is a lot of evidence to support that it is hazardous and pollutes the environment. According to the findings of studies, the biodegradation process, which makes use of bacteria, has the ability to restrict the amount of SDS that is released into the environment [1,2]. Because of their superior detergency at low temperatures in neutral solutions, anionic surfactants enjoy the greatest market share among all types. They are responsible for the production of ions in aqueous solution that are charged negatively and come from sulfate or sulphonate groups. Numerous commercial and industrial applications make extensive use of anionic surfactants, which include either ester sulfate or sulfonated groups derived from xenobiotic substances [3–13]. Due to the fact that they have a higher solubility of both organic and inorganic chemicals, they are an important group of organic substances that are found in SML. The exudates of phytoplankton that are found below the surface of the water are the primary source of naturally produced surfactants (SSW) [14]. Anionic surfactants are made up of hydrophilic and hydrophobic components, both of which are able to efficiently react with plar and apolar macromolecules substructures as well as polar and apolar entities in compound mixtures. Anionic surfactants are used in a wide variety of applications. As a result, anionic surfactants are able to bring benefits in a variety of heterogeneous phases in a wide variety of technological processes and biological systems by lowering the amount of energy required for contact and solvation [15]. Microorganisms able to degrade SDS and us it as a carbon source for growth and energy are the forefront of bioremediation of this hazardous compound in the environment [16–22].

Copper, second only to crude oil in importance, is a key raw commodity across the world. However, a considerable quantity of trash is produced throughout the copper processing procedure. Long-term copper exposure increases the aggregation and toxicity of -synuclein, which has been related to a variety of health problems, including growth and development issues, carcinogenesis, mental retardation, and Parkinson's disease . However, their extensive application causes them to disperse throughout the natural world. Each year, soils throughout the world are exposed to about 3.4 million tons of copper and 1 million tons of nickel. There are also concerning reports of the widespread pollution of farmland with sewage sludge, the primary source of heavy metals such as zinc, which exposes 20 million hectares of land to contamination from these xenobiotics. Copper's strong thermal and electrical conductivity and resistance to corrosion contribute to the metal's extensive use. Because of this, it finds widespread application in fields as diverse as electricity generation, electronics, machine and furniture production, and coinage [23].

Consuming acidic meals cooked on unprotected copper skillet or being subjected to excessive copper in drinking water or in food can lead to copperiedus (copper toxicity). Acute copper poisoning manifests itself in a variety of ways, with gastrointestinal symptoms being the most typical result of copper excess caused by ingestion. Many proteins and enzymes use copper as a cofactor for catalytic reduction and oxidation reactions, however free copper ions can be harmful to cells if they are present in excessive amounts. The quantity of copper in a cell is controlled by a finely tuned balancing act between its absorption and its outflow. Overexposure to copper results in oxidative stress, DNA damage, and a decrease in cell proliferation. When more than 1 gram of copper sulfate is consumed, hazardous effects begin to manifest. Primary copper toxicosis is caused by a genetic metabolic abnormality, while secondary copper toxicosis is caused by either excessive copper consumption, increased copper absorption, or decreased copper excretion [24–28].

Alterations in mental state, such as a headache or coma, as well as heart rate abnormalities, have been linked to gastrointestinal (GI) disorders. People with glucose-6-phosphate deficiency are more likely to experience negative hematologic effects of copper and patients with an intravascular mechanism of copper toxicity are at risk for copper toxicity and might present with signs/symptoms of intravascular hemolysis. Negative affective and behavioral outcomes, such as sadness, weariness, irritation, excitement, and inability to concentrate, are also recorded. Copper poisoning can cause rhabdomyolysis, methemoglobinemia, cardiac and renal failure, intravascular hemolysis, encephalopathy, liver necrosis and mortality in its most severe forms [24–26,29,30].

The occurrence of hazardous metal ions in contaminated effluent had the effect of stifling the growth of bacteria as well as limiting their ability to make use of toxic substances like SDS. Because of the effect they have on the microorganisms that are responsible for breaking down pollutants, the noxiousness of high-saline environments and the simulataneous presence of heavy metal ions has received a lot of attention in recent years. This is due to the fact that they either inhibit the growth of the microorganisms or decrease the activity of the enzymes that the microorganisms produce. There are certain physically based methods that are capable of removing salts, including as osmosis, ion exchange, and dialysis; however, the cost of using these techniques in an industrial setting is prohibitively expensive. There are some metals that cannot even be tolerated at slightly increased concentrations in the environment, despite the fact that there are some species that are able to withstand heavy metals and even reduce their levels. These metals include those that are found in nuclear waste. Chelating the metal ions that are the root cause of the inhibition that these metals induce is one way to lessen its impact and make it more manageable. Precipitation, sorption, and chelation of heavy metals by organic and inorganic ligands are the three most important strategies for lowering the toxicity of heavy metals. Precipitation can be achieved through precipitation, sorption, and chelation of heavy metals.

If biodegradation is slowed by heavy metals, bioremediation may take longer to complete. Heavy metal ions, unlike many other inhibitors, cannot be destroyed, therefore once they have been accumulated by microbes to a toxic quantity, they restrict the growth rate of the bacteria. This is because heavy metal ions cannot undergo degradation. Therefore, the inhibitory characteristics caused by the presence of harmful ions may be studied by making adjustments to the model of substrate inhibition. Numerous models, including a modified version of the Han-Levenspiel [31], Liu [32], Wang [33], modified Amor [34], Andrews [35] and the Shukor model [36] 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.

These studies are almost the only ones available at this time that examine the impact of heavy metals on microbial development. This is because most studies on the impact of metals on microbial development use primary models rather than secondary models. An SDS-degrading bacteria was isolated, and it was observed that heavy metals including mercury, silver, and copper significantly inhibited its growth [37,38]. By employing a number of different inhibition models, the purpose of this work is to investigate the impact that copper 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 [39]. Studies on the effect of heavy metal to the growth of the bacterium on SDS utilized the microtiter plate format [40,41]. 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}) [37]. 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).

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Growth model on SDS

Since the modified Gompertz model is the one that is typically utilized for modeling the growth of microorganisms on xenobiotics, this model was chosen in order to predict the highest specific growth rate that might be achieved on SDS [42–44]. The equation is as follows;

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

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	$r = u_{max}\left(1-\frac{C}{C_{crit}}\right)^m$	[31]
Wang	$r = \frac{u_{max}}{1+\left(\frac{C}{K_C}\right)^m}$	[33]
Liu	$r = \frac{u_{max}K_C}{K_C + C}$	[32]
Modified Andrews	$r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[35]
Shukor	$r = v_{max}\left(1-\left(\frac{C}{S_m}\right)^n\right)$	[36]
Amor	$r = \frac{u_{max}C}{C + \left(\frac{C^2}{K_i}\right)}$	[34]

Fitting of the data

In order to fit the nonlinear equations, a Marquardt approach was used, and the CurveExpert Professional program was utilized (Version 1.6). The algorithm looks for the approach that will result in the lowest possible sum of squares between the values that were predicted and those that were actually measured. The initial values are automatically calculated by the software using the method that takes into account the sharpest ascent.

Statistical analysis

Numerous statistical methods, such as 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) were applied as before in order to select the optimal model. [45].

RESULTS AND DISCUSSION

The anionic surfactant sodium dodecyl sulfate (SDS) has many applications and is often discarded via the environment via water and soil. Due to its toxicity, which may kill species, SDS must be eliminated from the environment. Therefore, experts recommend bioremediation as a viable strategy for lessening SDS toxicity [46].As a result of their efficiency and low price, bacteria are the preferred method for cleaning up SDS contamination in contaminated soils and water sources at the moment. Increases in SDS disposal need for the use of superior strains to assure environmental safety [47]. According to research by Rebello et al., SDS breakdown is often carried out by *Pseudomonas* species in soil or aquatic environments. The ubiquitous Pseudomonas genus is a common and potent degrader of hazardous chemicals, making it useful in reducing the impact of industrial effluents, particularly sodium dodecyl sulfate (SDS) [48]. The growth of the bacterium at different doses of copper demonstrates a sigmoidal pattern, with lag periods varying from seven to ten hours (**Fig. 1**). The total growth was impeded when the concentration of copper was increased, with a concentration of 1.0 g/L causing the growth to practically come to a complete halt. The modified Gompertz model was applied (**Fig. 2**) in order to obtain growth rates at various concentrations of copper. The model demonstrates a close fitting to the data, which is consistent with the model's predictions. The model also demonstrates that an increase in the concentration of copper resulted in a reduction in growth rates and an increase in the lag time. This was the case even if the lag period lengthened.

Fig. 1. Growth of *Serratia marcescens* strain DRY6 on SDS at a concentration of 1.0 g/L SDS in the presence of copper at several concentrations ranging from 0.2 to 1.0 mg/L. The mean and standard deviation of the triple measurements are depicted by the error bars.

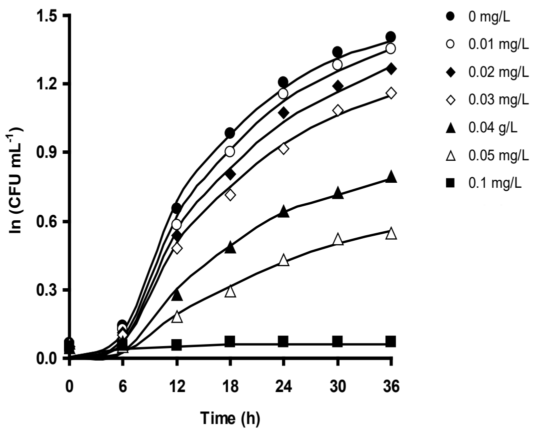

Fig. 2. Growth of *Serratia marcescens* strain DRY6 on SDS at a concentration of 1.0 g/L SDS at different concentrations of copper (ranging from 0.2 to 1.0 mg/L) as modeled using the modified Gompertz model. Growth is shown as a log transformation.

After that, the growth rates at a range of different copper concentrations were modeled using the various metal inhibition models that were available. With the exception of the Amor model, all of the other models were able to fit the curve. The Amor model, however, was not able to fit the curve (**Figs. 3** to **5**). In contrast, the Wang and modified Han-Levenspiel models exhibit fitting that is acceptable, whereas the Liu model displays fitting that is unacceptable. The statistical study revealed that the Wang model was the most accurate representation of reality, since it produced the fewest outliers.for RMSE and AICc, 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

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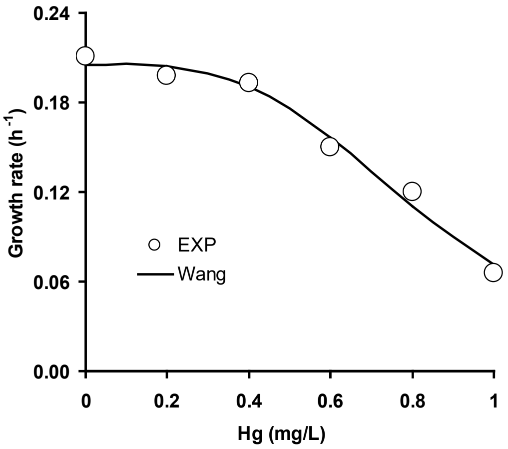
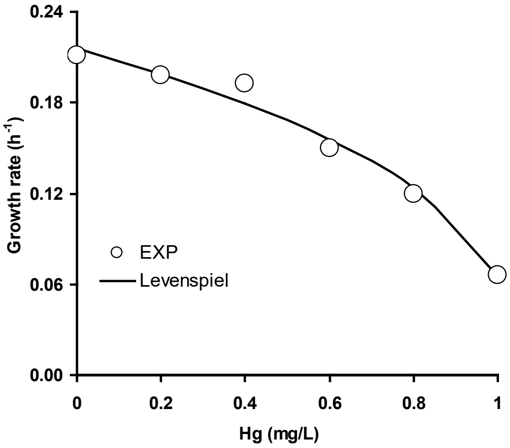
Note:
p no of parameter
adR^2 adjusted correlation coefficient
RMSE Root mean square error
AF Accuracy factor
BF Bias factor
AICc corrected Akaike Information Criteria
n.a. not available





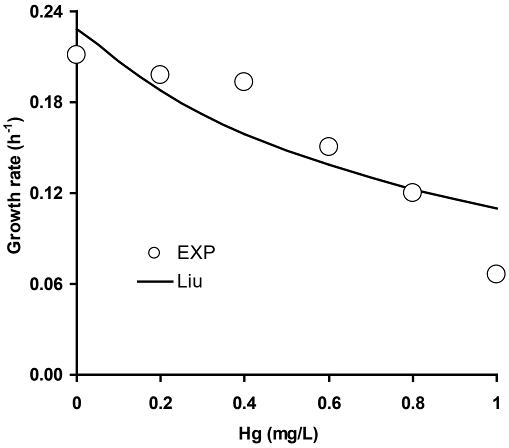


Table 2. Parameter values for growth rate inhibition models.

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

The results of using the Shukor model yielded the following values for the parameters C_{crit}, μ_{max} and *m* of 0.209, 0.103, and 1.530, respectively. These values reflect the critical heavy metal ion concentration (mg/L), maximum growth rate (per h), and empirical constant values, respectively. The Shukor model enables the prediction of the critical heavy metals concentration that can fully suppressed bacterial development, which is highly valuable in translating laboratory data to the field. Moreover, the Shukor model can be found here.

Few studies have examined the effect of heavy metals on the population increases of bacteria living on toxic compounds. For examples, zinc and nickel drastically lowered the rate of biodegradation of monoaromatic hydrocarbons by Bacillus sp. and Pseudomonas sp., and the Andrews model is successfully used to study the impact of these heavy metals on the degradation rate [26]. Heavy metals are thought to decrease enzyme activity by binding to critical enzyme functional groups like the sulfhydryl group, which is frequently located in the active regions of enzymes [34]. This is presumably the mechanism behind this inhibition of biodegradation. Notwithstanding the requirement of these research given the presence of heavy metals in organically polluted streams, the use of metal inhibition models is underrepresented in the scientific literature. It is believed that heavy metals inhibit enzyme activity by attaching to essential enzyme functional groups, such as the sulfhydryl group, which is typically found in the active areas of enzymes. Presumably, this is the mechanism responsible for this inhibition [49].

There are a number of different approaches that may be taken to address the problem of heavy metals preventing biodegradation. Calcium carbonate, manganese oxide, cement, phosphate, and magnesium hydroxide are all examples of treatment additives that can limit the bioavailability and mobility of metals, making it simpler to clean up metal pollution [50]. One alternate approach is to make use of the minerals found in clay. Clay minerals have been demonstrated to be effective at lowering the bioavailability of metals as well as the toxicity that follows from the presence of metals in an environment. The toxicity of cadmium was reduced, for example, when kaolinite (1-20 percent) or montmorillonite (1-5 percent) was added to agar media containing cadmium for use by yeasts, bacteria, and an actinomycete. The cadmium-containing agar medium was used by yeasts, bacteria, and an actinomycete [51]. Similarly, Kamel (1986) discovered that 3 percent bentonite and vermiculite in solution testing reduced the toxicity of 150 mg total cadmium/L to *Streptomyces bottropensis*. In spite of the fact that kaolinite was successful in reducing the toxicity of cadmium, it demanded

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a larger concentration (six percent as opposed to three percent) and offered less protection all around than the other clays [52]. Inoculation of metal-resistant bacteria is another approach that may be used. This reduces the amount of bioavailable metal in the environment, which speeds up the process of biodegradation when a hazardous metal is present. Inoculation of metal-resistant bacteria is another approach that may be used. This reduces the amount of bioavailable metal in the environment, which speeds up the process of biodegradation when a hazardous metal is present [53]. It is possible to improve the effectiveness of acrylamide breakdown by combining a primary bacterial degrader with a bacterium that is resistant to metals. A cadmium-resistant *Pseudomonas* H1, which accumulates cadmium in the cell, and 2,4-D-degrading bacteria were introduced to soil that was contaminated with both cadmium (60 mg total cadmium/kg) and 2,4-D (500 mg/kg), which resulted in a better degradation efficiency of the xenobiotic. This is shown as an example in a soil microcosm experiment study [54].

CONCLUSION

To sum up, despite the importance of the study, metal inhibition models are seldom used to predict the effect of metal ions on the growth rate of bacteria exposed to hazardous substances. Despite the importance of such research, this has not happened. Several metal inhibition models were used to predict how copper would affect the growth of bacteria capable of digesting SDS in this study. It turned out that the Wang model was the best at describing the phenomena. By using the Wang model, we can estimate the critical concentration of heavy metals required to completely halt bacterial growth. It's realistic to assume that the pace of development on harmful substances will be considerably impacted by the presence of heavy metals. Because the bacteria will need to be able to survive the toxicity of both types of toxicants concurrently, this is the case. Incorporating SDS bioremediation into copper-polluted areas is the goal of future field trial initiatives, and the results of this study may prove valuable in such endeavors.

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